STUDY OF EFFICACY AND TOLERABILITY OF TOPICAL VORICONAZOLE VERSUS NATAMYCIN IN THE TREATMENT OF FILAMENTOUS FUNGAL KERATITIS

DISSERTATION SUBMITTED FOR THE DEGREE OF

M.D BRANCH –VI

PHARMACOLOGY

APRIL – 2018

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY,

CHENNAI - 600 032

TAMILNADU

Madurai

.10.2017

CERTIFICATE

This is to certify that the dissertation entitled **"A STUDY OF EFFICACY AND TOLERABILITY OF TOPICAL VORICONAZOLE VERSUS NATAMYCIN IN THE TREATMENT OF FILAMENTOUS FUNGAL KERATITIS** is a bonafide record of work done by **Dr.S.PRASANNAKUMARI**, under the guidance and supervision of **Dr.M.SHANTHI, M.D.**, Professor, in the Institute of Pharmacology, Madurai Medical College, Madurai during the period of her postgraduate study of M.D Pharmacology from 2015-2018.

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DECLARATION

I, **Dr.S.PRASANNAKUMARI** solemnly declare that the dissertation entitled **"A STUDY OF EFFICACY AND TOLERABILITY OF TOPICAL VORICONAZOLE VERSUS NATAMYCIN IN THE TREATMENT OF FILAMENTOUS FUNGAL KERATITIS"** has been prepared by me under the able guidance and supervision of **Dr.R.PARAMESWARI, M.D,** Director and Professor, Institute of Pharmacology, Madurai Medical College, Madurai, in partial fulfillment of the regulation for the award of M.D Pharmacology degree examination of The TamilNadu Dr.M.G.R Medical University, Chennai to be held in April 2018.

This work has not formed the basis for the award of any degree or diploma, previously from any other university to me or anyone.

Place: Madurai **Dr. S.PRASANNAKUMARI**

Date:

ACKNOWLEDGEMENT

I am greatly indebted to **Dr.D.MARUTHUPANDIAN, M.S., FICS., FIAS,FAC** Dean, Madurai Medical College and Govt Rajaji hospital, Madurai who initiated this interdisciplinary work with generous permission.

It is with great pleasure I record my deep respects, gratitude and in debtedness to **Dr.R.PARAMESWARI,** M.D., Director and Professor, Institute of Pharmacology, Madurai medical college, Madurai for her remarkable guidance, encouragement and selfless support which enabled me to pursue the work with perseverance**.** Her contagious enthusiasm was a source of energy to me in successfully completing my dissertation under her generous guidance.

I am extremely thankful to my guide **Dr.M.SHANTHI,** M.D., Professor, Institute of Pharmacology, Madurai Medical College, Madurai, for her valuable suggestions and critical review at every stage for the successful completion of this study.

I record my sincere and heartfelt thanks to **Dr.S.VIJAYALAKSHMI,M.D.,** Professor of Pharmacology, Madurai Medical College, Madurai for her untiring support, continuous suggestions and enduring encouragement throughout the study.

I record my sincere and heartfelt thanks to **Dr.R.SAROJINI, M.D.,** Professor of Pharmacology, Madurai Medical College, Madurai for her untiring support, continuous suggestions and enduring encouragement throughout the study.

I am thankful to **Dr.K.RAADHIKA, M.D.,** Associate Professor of Pharmacology, for her valuable suggestions and support.

I wish to express my sincere thanks to **Dr.P.THIYGARAJAN, M.S,DO., & Dr.S.V.CHANDRAKUMAR, M.S.,DO.,** Head of Department, Department of Ophthalmology, Government Rajaji Hospital, Madurai for the generous permission and complete co-operation to carry out the study.

I express my heartful thanks to **Dr.P.A.T.JEGATHEESWARY, M.D,** & **Dr.R.VIBUSHANAN, M.D.,** Professor, Institute of Microbiology, Government Rajaji Hospital, Madurai for their immense help during this study.

It is with a deep sense of gratitude, I wish to express my sincere thanks to Assistant Professors **Dr.M.SHEIKDAVOOTH, M.D., Dr.V.THAIVANAI, M.D., Dr.M.S.AHIL, M.D., Dr.K.GEETHA, M.D**., **Dr. M.MALATHI, M.D., Dr.B.JAYAPRIYA, M.D., Dr.GOWRITHILAGAM, M.D.,** and **Dr. SHANKRESHWARI, M.D.,**

I am extremely thankful to **Dr.M.SALEEM, M.D.,** and **Dr. PRIYA, M.D.,** Associate Professor, Institute of Preventive and Social Medicine, Madurai Medical College, Madurai for their valuable suggestions and support for the statistical work.

It is my duty to express my appreciation to my colleague **Dr.S.VASANTH, Dr.R.VIJAYARANI, Dr.G.MUTHUKAVITHA, Dr.C.UMAMAHESHWARI, Dr.R.MANGALADEVI, Dr.S.KIRUTHIKA, Dr.X.APRASANNA, Dr.M.PRIYALAKSHMI, Dr.V.SIVARANJAINI, Dr.K.PORCHELVI, Dr. R.THEIVANAYAGI, Dr.V.VINOTHINI** and **Dr.S. MEENAMBAL** for their assistance.

I thank my family members and staff members of the Institute of Pharmacology for their kind support and encouragement throughout the study.

I thank the almighty who had bestowed his mercy and kindness all throughout my study and my carrier.

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INTRODUCTION

INTRODUCTION

The most pathetic person in the world is someone

who has sight but no vision".

-Helen Keller

A good vision is a fundamental necessity in attaining a meaningful and fulfilling life. A clear vision helps us to pursue dreams and achieve goals. When there is no vision, there is no direction. But despite remarkable advances in medical field visual impairment and blindness remain a major public health problem.

Visual impairment also known as vision impairment or vision loss is a decreased ability to see. Visual impairment is often defined as a best corrected visual acuity of worse than either 20/40 or 20/60.[1] Bilateral visual impairment may cause difficulties in normal daily activities such as reading, driving, socializing and walking.[2]The term blindness is used for complete or near complete vision loss.

The estimated prevalence of blindness in 1990 ranged from 0.08% of children to 4.4% of persons aged over 60 years with an overall global prevalence of 0.7%[3]. It was estimated that the extent of visual impairment in global population is likely to increase from 5.8 billion in 1996 to 7.9 billion by 2020.

Global causes of blindness due to eye diseases, excluding refractive errors are cataract 47%, glaucoma 12%, AMD(Age related macular

degeneration) 9% and corneal opacities 5%.The opacities are mainly due to infection of the cornea like aberrations and ulcers.[4]

Corneal ulcer is defined as loss of corneal epithelium with inflammation in the surrounding stroma. Keratitis is the term used for any type of corneal inflammation.

The types of corneal ulcer are,

- Baterial
- Fungal
- Viral
- Acathomoebal

Though bacterial keratitis accounts for 80% of corneal ulceration, fungal keratitis is more virulent and is considered as major cause of worse visual outcome with higher rates of corneal perforation and monocular blindness in the developing world[5]. Early diagnosis and proper treatment may reduce the morbidity of the disease. The fungal infection is unique in many ways like etiology, presentation, diagnostic tests, response to treatment and their sequelae also differ from other corneal infections.

Historically, fungal keratitis has been endemic in warmer climates such as India, and has been relatively uncommon in temperate regions. It is due to the fact that, the main working populations in such countries is agricultural, where they are exposed to vegetable matter and other organic contaminants which is considered as a main cause of fungal infection of the eye. The secondary causes includes immune- compromised individuals, post - operative

cases, injudicious usage of antibiotic/steroid drops, use of traditional home made contaminated medication and the alarmingly increasing trend of self medication have increased the incidence of fungal infection.

Fungi are ubiquitous organisms is recognized as most common ocular pathogens in tropical countries. There are over 100,000 species of fungi. The specific ocular pathogenic fungi include *Aspergillus , Fusarium, Curvularia* and *Candida*.[6]

Fungal keratitis usually has an indolent course. The signs will be more severe than symptoms. Definite diagnosis requires laboratory confirmation by scraping the ulcer base for staining and culture. The antifungal should be instituted at the earliest following the availability of the smear report[7]. The early diagnosis and treatment will prevent the complications and visual impairment.

The pharmacological agents used for fungal keratitis include the antifungals like Polyene, Imidazoles, Pyrimidines, Triazole and many others.However the efficacy of these drugs is limited. Natamycin has been the first line drug for the treatment of superficial filamentous fungal keratitis for decades and is the only antifungal eye drops commercially available.[8]

Recent studies have suggested that Voriconazole can be a suitable alternative to Natamycin as it is active against *Candida* and also filamentous fungi. [9]There are only limited studies comparing the efficacy of these drugs, so an attempt was made to study the efficacy and side effect profile of Azoles and Polyenes in patients with filamentous fungal corneal ulcer attending

Ophthalmology Department of Government Tertiary Care Hospital. Natamycin and Voriconazole are the most commonly used drugs in the Ophthalmology department for treating fungal keratitis, so they were selected to be the candidate drugs and their efficacy, tolerability and safety were analyzed in this present study.

AIM

AND

OBJECTIVE

AIM AND OBJECTIVE

To study the efficacy, tolerability and safety of topical **Voriconazole** and **Natamycin** ,in patients with superficial filamentous fungal keratitis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cornea is a transparent, avascular tissue with smooth surface. It comprises one-sixth of the anterior eyeball. The cornea act as a protective membrane, as well as strong refractory surface. It is the most densely innervated tissue in the body. Histologically cornea consist of six layers,

1. Epithelium
2. Bowman's membrane
3. Stroma (substantia propria)
4. Pre- Descemet's membrane
5. Descement's memberane
6. Endothelium

The transparency of the cornea is mainly due to its peculiar lamellar arrangement of collagen fibers, selective permeability of the epithelium, avascularity and deturgescence. The corneal deturgescence is maintained by active sodium-potassium pump in the endothelium.[10]

The main function of cornea includes,

- ❖ Transmission of light by its transparency
- ❖ Focus light by refraction
- ❖ Maintaining the structural integrity of the globe
- ❖ Protecting the eye from infections, UV radiation and noxious substances

Cornea being exposed to the external environment is highly prone to atmospheric irritants such as smoke, dust, heat, dry air and sand that affects the ocular surface. The conditions such as abrasions and ulcers are usually associated with marked pain, photophobia and reflex lacrimation. [11]

The inflammation of cornea is known as keratitis. Infectious keratitis is a broad term for corneal diseases caused by infective agent .The inflammatory conditions of the cornea may arise from,

Exogenous infection:

- Direct invasion of the organism (bacterial/fungal/viral)

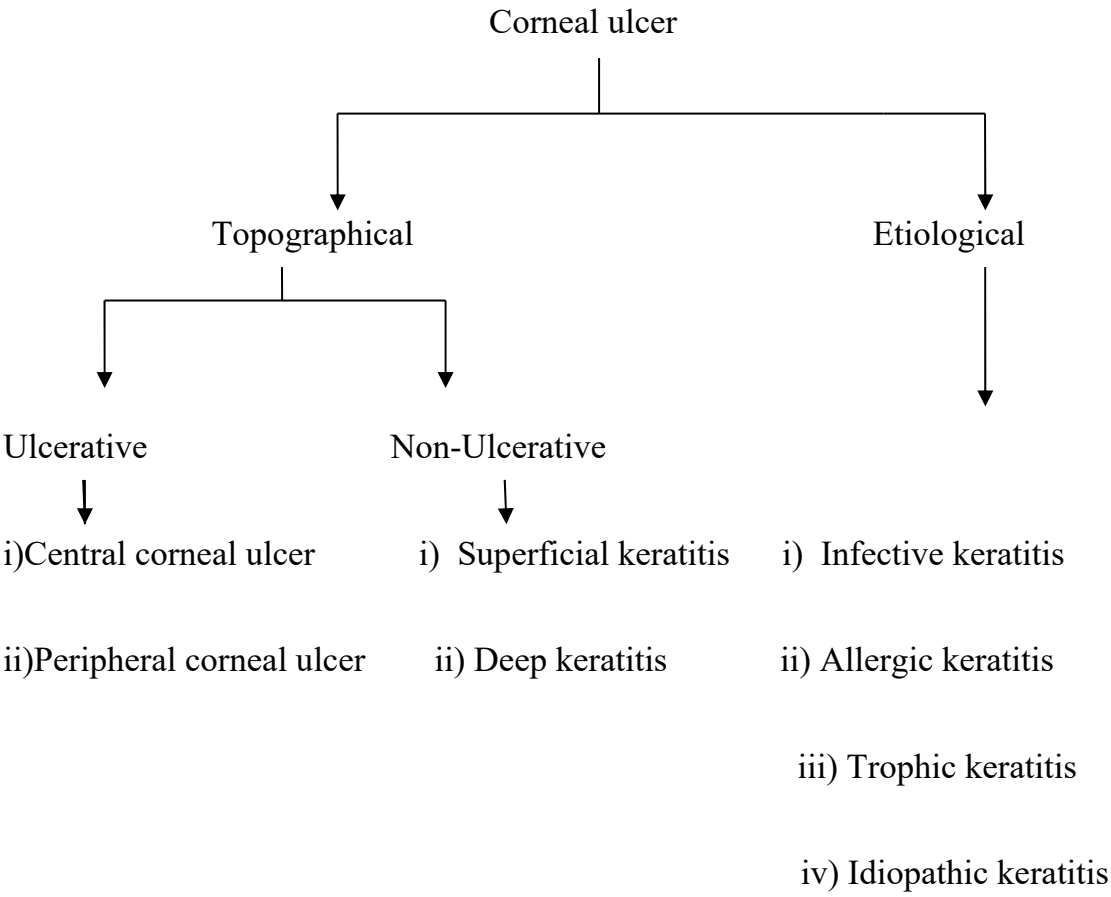
Endogenous infection:

- Associated with systemic, allergic and hypersensitivity reactions.

Secondary infection:

- Due to disease of conjunctiva, sclera and uvea.

Corneal inflammation can be classified as[12] ,



Infective keratitis is further classified as

- Bacterial keratitis
- Viral keratitis
- Fungal keratitis
- Chlamydial keratitis
- Spirochaetal keratitis

Bacterial keratitis:

Infection of the cornea is always exogenous due to pyogenic bacteria such as Pseudomonas, Staphylococcus, Pneumococcus, Neisseria gonorrhoea or Escherichia coli.

Fungal keratitis:

Mycotic keratitis is commonly due to *Aspergillus, Fusarium* or *Candida albicans.*

Viral keratitis:

Viral keratitis is mainly due Herpes zoster, Adenoviruses and Chlamydia trachomatis and it usually causes superficial keratitis.

Acanthamoeba keratitis:

It is a protozoal keratitis caused by Acanthamoeba castellani, a free living amoeba found in soil, fresh water, well water, sea water, sewage and air. Contact lens wearers are more prone for this keratitis.

PATHOGENESIS OF CORNEAL ULCER:

The pathological changes that occur during development of corneal ulcer is described under four stages[13],

1) Stage of infiltration
2) Stage of active ulceration or progression
3) Stage of regression
4) Stage of cicatrisation

- Intact corneal epithelium is an important defence factor
- When the infective organism overcomes the host defence
- Penetration of the organism occurs

↓

- Lymphocyte infiltrates in the epithelium
- Necrosis

STAGE OF PROGRESSIVE INFILTRATION

↓

- Greyish infiltration with circumcorneal Hyperaemia
- Hypopyon and descemetocele formation

STAGEOF ACTIVE ULCERATION

↓

- Phagocytosis
- Ulcer begins to heal

STAGE OF REGRESSION

↓

- Epithelium covers the ulcers
- Scares and opacities formation

STAGE OF CICATRISATION

FUNGAL KERATITIS :

Fungal keratitis was first described by Leber in 1879[14].Fungal keratitis (keratomycosis) is a fungal infection of the cornea. It primarily affects the corneal epithelium and stroma but in severe infections anterior chamber and endothelium of the eye may get involved. Fungal keratitis is an opportunistic infection of the eye, they rarely infect intact and healthy cornea (unlike other bacteria species like Neisseria gonorrhoea).

ETIOLOGY OF FUNGAL CORNEAL ULCER :

Young healthy adults (21-60 years) are affected by fungal ulcer, who live in rural area. Males are commonly affected, where the main occupation is agriculture.[15]

Common risk factors for corneal ulcer includes,

1. Trauma- Injury with vegetable matter
2. Prolonged contact lens wear[16] } 4- 30%
3. Topical steroid use[17] }
4. Long term use of antibiotic
5. Immunocompetent individuals
6. Iatrogenic[18]
   - Following cataract surgery
   - Penetrating keratoplasty
   - Laser-assisted in situ keratomileusis [LASIK]
7. Other ocular factors[19]
   - Corneal surface disorder
   - Allergic conjunctivitis

8. Systemic diseases[20]

Diabetes - 5%

Malnutrition - 1%

Alcoholism - rare

HIV - rare

EPIDEMIOLOGY:

Fungal keratitis is a major disease causing blindness in Asia. Depending on the geographic locations, its incidence is between 6- 20%. It has also been shown that fungal keratitis (FK) is more virulent and damaging compared to bacterial keratitis.

The prevalence of fungal pathogens in South India is significantly greater. Report from South India have found that about 44% of all corneal ulcers are caused by fungi and the incidence is,[21,22,23]

- Nepal - 17%
- Bangladesh- 36%
- Ghana - 37.6%

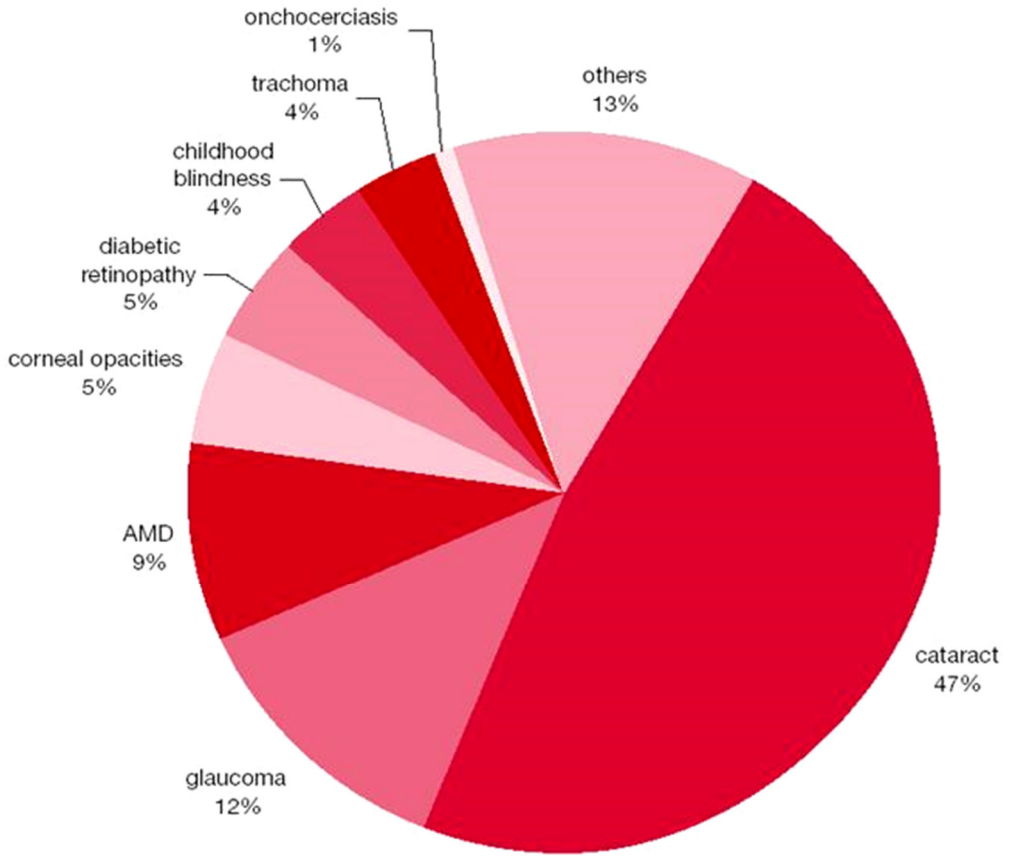
Common species that causes fungal infection includes[24],

In India,

- Aspergillus → 27 – 64%
- Fusarium → 6- 32 %
- Pencillium → 2- 29%

According to WHO estimates in 2006, there will be approximately 314 million people around the world with vision impaired, either due to eye diseases or uncorrected refractive errors. Out of this about 45 million people will be blind. According to this statistics corneal scar accounts for 5% of total blindness, and this scar is mainly due to corneal ulcer (Bacterial, Fungal).

Figure 1 Global causes of blindness due to eye diseases, excluding refractive errors



FUNGUS:

Fungi are eukaryotic plant like micro organism that occur in environment. There are over 250,000 species of fungi but few are human pathogens. Opportunistic fungi are harmless commensal seldom produce pathological lesions.

BROAD GROUPS OF FUNGI PATHOGENIC TO EYE:

The fungi, pathogenic to the eye include[25]

- **Filamentous fungi or Moulds :**
  - Septate (Hyphae) :

    Nonpigmented - *Aspergillus or Fusarium*

    Pigmented - *Alternaria or Curvularia*
  - Nonseptate organism *:*

    Mucor
- **Yeasts:**

  Candida
- **Dimorphic Fungi**

  Blastomyces , Cryptococcus , Sporothrix.

The most common organism causing fungal keratitis is filamentous fungi , which includes *Aspergillus* and *Fusarium* species. As per the clinical studies conducted by Lalitha et al out of 100 fungal isolates, 41 is *Fusarium*, 32 is *Aspergillus flavus*, 18 is *Aspergillus fumigatus.*

FUSARIUM :

It is the most important plant pathogen, prevalent in crop plants[26]. It causes localised lesion after trauma in healthy individuals. *Fusarium* is microscopically identified by its sickle or banana shaped macrocondia[27].

RATE OF GROWTH:

They are very rapid growers, mature within 4 days.

COLONY MORPHOLOGY:

Colonies are white and cottony, but quickly develop into a pink or violet center.

MICROSCOPIC MORPHOLOGY:

Septate hyphae contain two types of conidiation,

i) Unbranched or branched conidiation that produce cone shaped macroconidia

ii) Long or shorter conidiation bearing small or oval conidia in clusters or single.

Figure : 2 FUSARIUM

Culture media ***Microscopic view***

ASPERGILLUS :

Members of genus *Aspergillus*, causes group of disease known as aspergillosis. They are commonly found in decaying vegetation and soil[28]. Infection in human were initially described by Micheli in 1729.They are major pathogens of human and causes invasive diseases in the cornea. Although there are 200 species, 95% of all *Aspergillus* infections are caused by *Fumigatus. Aspergillus* species produce potent proteolytic enzymes such as elastase.

RATE OF GROWTH:

Most of the species are rapid grower and mature within 3 days.

COLONY MORPHOLOGY:

Surface of the colony is white to any shades of green, yellow, orange, brown or black depending on the species. The texture is velvety or cottony.

MICROSCOPIC MORPHOLOGY:

Hyphae are septate about 2.5-8μm in diameter. From a specialized foot cell an unbranched conidiophore arises. The conidiophores contain vesicle enlarged at the tip. Vesicles are completely or partially covered with flask shaped phialides[29].

Figure : 3 *ASPERGILLUS FLAVUS*

Culture media *Microscopic view*

Figure: 4 *ASPERGILLUS FUMIGATUS*

Culture media *Microscopic view*

SYMPTOMS AND SIGNS OF FUNGAL CORNEAL ULCER:

SYMPTOMS:

It is usually non specific and prolonged

- ❖ Foreign body sensation
- ❖ Slow onset of increasing pain

- Diminution of vision
- Watering
- Redness of eye
- Photophobia

SIGNS:

Non specific :

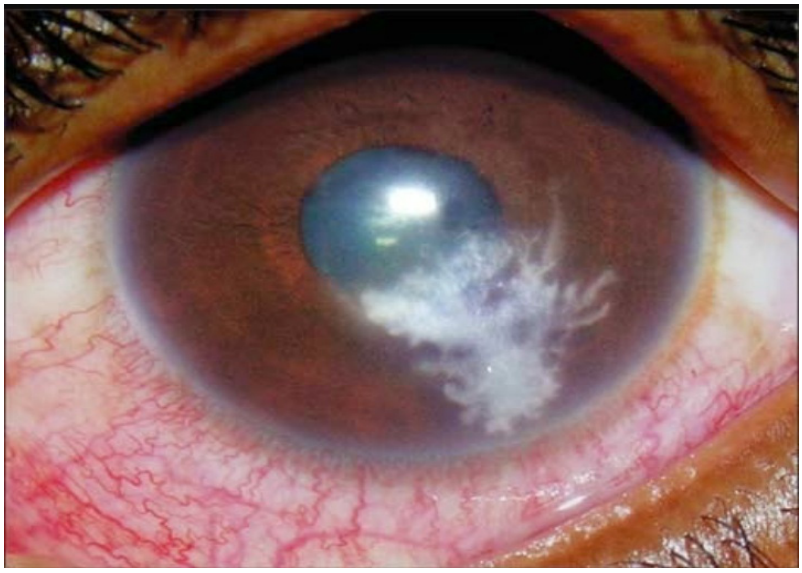
- Conjunctival injection
- Epithelial defect
- Anterior chamber reactions

Specific :

The salient features of fungal corneal ulcer are,

- Cornel ulcer is dry looking, greyish white with elevated rolled out margins.
- Feathery finger like extensions
- A sterile immune ring (yellow line of demarcation) – where fungal antigen and host antibody meet
- Single or multiple satellite lesion (may or may not be present)
- Hypopyon - usually big even if the ulcer is small. Non sterile, thick &immobile.

Figure : 5 **FUNGAL CORNEAL ULCER**



Corneal scraping is the ideal sample to demonstrate the presence of fungi in corneal ulcer.[30]

PROCEDURE FOR SCRAPING :

- Scraping should be done under slit lamp/operating loupe/ operating microscope.
- Instill 1-2 drops of topical anaesthetic agent.
- Wait for 1 min. Keep 2 clean glass slides having 1cm circle with glass pencil on reverse side of slide.
- Scrape base and edges of corneal ulcer with flame sterilized kimura spatula or sterile 15# BP blade.
- Streak over glass slide within circle for stains.

STAINING TECHNIQUES:

Commonly used stains are

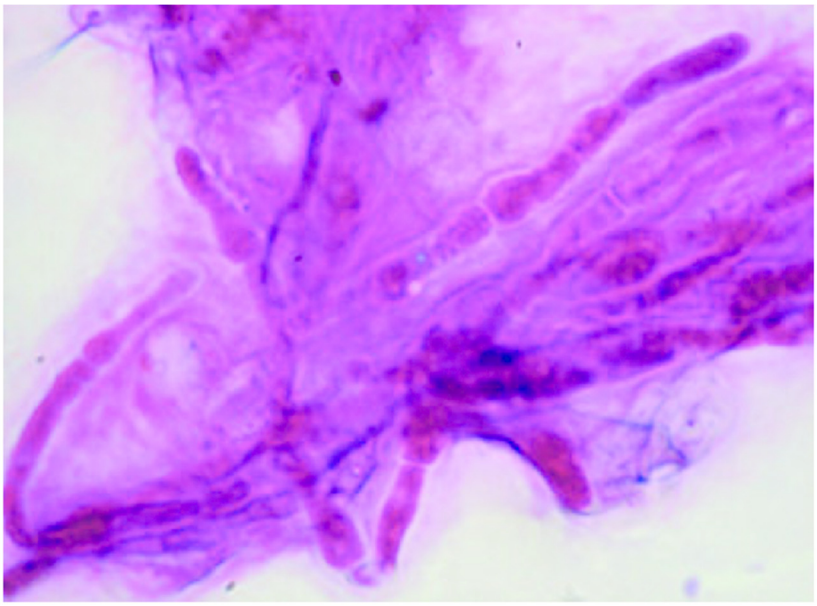
- Gram stain
- 10 % KOH mount.

- Gram stain procedure,[31]

  - Fixation with methyl alcohol
  - Flood with crystal gentian violet
  - Rinse with water
  - Flood with Grams iodine
  - Rinse with water
  - Decolourise with acid alcohol
  - Rinse with water
  - Counter stain with safranine
  - Rinse with water

Readily shows the budding yeast of *candida* species. But fail to stain hyphae of moulds. They stain the walls of the fungi.

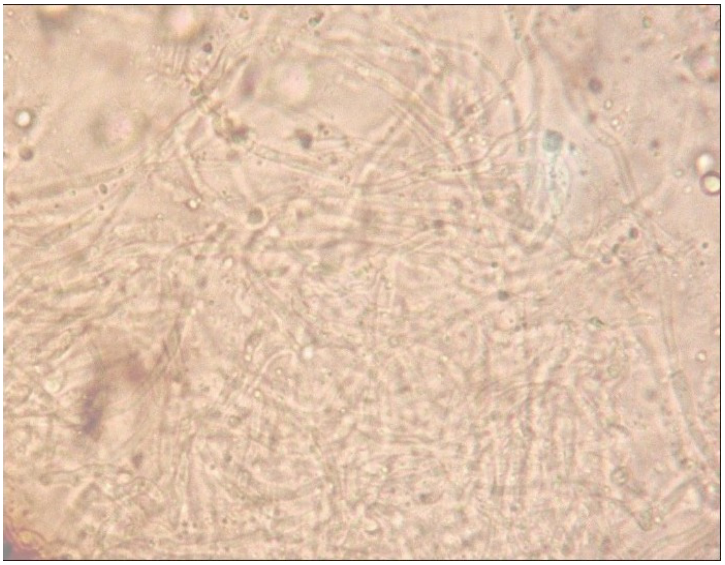
Figure: 6 **GRAM STAIN**



- 10% KOH mount preparation,[32]
    - 10 g of KOH
    - Dissolve in 10 ml of water
    - Add one drop of 10%glycerol

Apply KOH and Gram stain over the specimen cover with cover slip and examine under light microscope for the presence of fungal hyphae.

Figure: 7 **10% KOH MOUNT**



10 % KOH mount with fungal hyphea

Other commonly used stains are [33]

- Gomori-methenamine sliver nitrate
- Ink potassium hydroxide
- Periodic Acid – Schiff (PAS)
- Acridine orange
- Calcoflur white

CULTURE MEDIAS:

The fungal culture media used are,

SABOURAUD DEXTROSE AGAR:

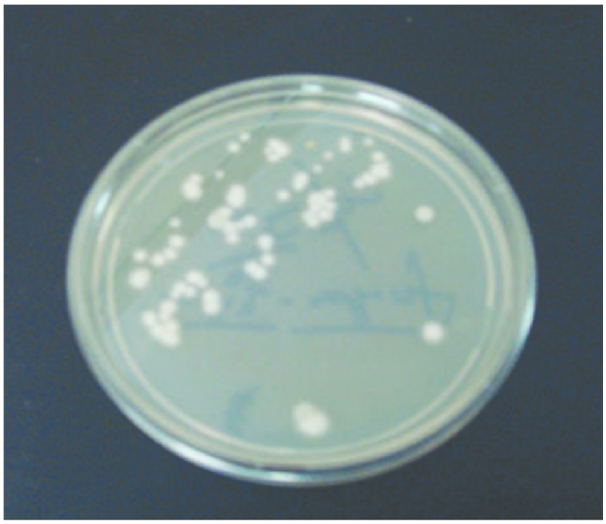
The fungal specimens should be incubated at 25◦C to 30◦C. Moulds formafuzzy colonies on culture plates. Pigmentation of mould depends on the culture media[32]. On Sabouraud dextrose agar, the colonies of

Aspergillus fumigatus - Blue – green

Aspergillus flavus - Yellow-green

Aspergillus niger - Black

Figure : 8 **SD AGAR**



Other media used for fungal isolation are,

- Chocolate agar
- Thioglycolate broth
- Brain heart infusion (BHI)

Other modes of investigation are,

- Polymerase chain reaction
- Confocal microscopy
- Corneal biopsy

The examination of corneal ulcer include,

SLITLAMP EXAMINATION:

Slit lamp bio-microscopy is used to visualize the ocular structures. It is a high powered binocular compound microscope with a slit shaped illumination source for the detailed examination of the anterior segment of the eye.

Due to its dynamic flexibility, it permits examination of the cornea with wide magnification and illumination. The bio-microscope provides the magnified three dimensional views that can be observed and photographed. The slit lamp illuminator was originally fashioned by Gullstrand[34].

Slit lamp examination is mainly used for the detailed examination of ulcer characters like ,

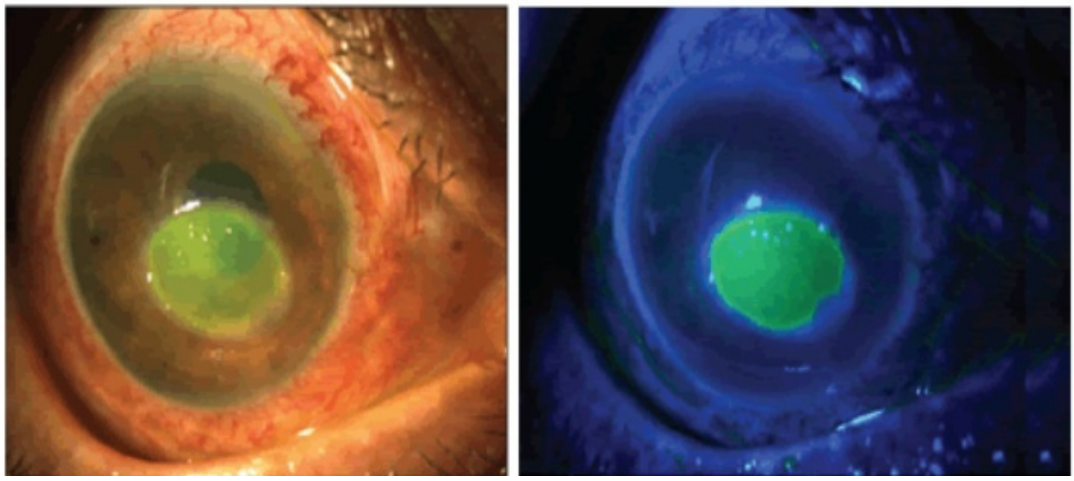
1. Size of epithelial defect
2. Depth of infiltration and stromal involvement
3. Sclera involvement
4. Anterior chamber (AC) reactions
5. Hypopyon size, satellite lesions & immune ring

Figure : 9 **SLIT LAMP BIOMICROSOPY**

1. SIZE OF EPITHELIAL DEFECT :

Epithelial defect can be assessed by staining methods. Fluorescein dyes are commonly used. Fluorescein stains the areas where the epithelial cells are missing. It is performed by topical application of dye either with fluorescein strips or with freshly prepared 2% solution of sodium fluorescein. High concentration of fluorescein is readily visible with white light and for the detailed examination 'Cobalt Blue' filter is used . The epithelial defects appear as yellow (exited fluorescein) against blue back ground. The defect can be measured in millimetres.

Figure : 10 **FLUORESCEIN STAIN**



2. DEPTH OF INFILTRATION AND STROMAL INVOLVEMENT :

Stromal infiltration is classified into,

- Superficial
- Mid stromal
- Deep or total corneal stromal thickness involvement

The depth of ulcer is categorized as

- 0% - 33% Mild
- 33 % - 66% Moderate
- 66% - 100% Severe

3. SCLERAL INVOLVEMENT :

Presence or absence is detected.

4. AC REACTION :

The presence of aqueous cells and flares indicates the acute inflammation of cornea, resulting in iritis.

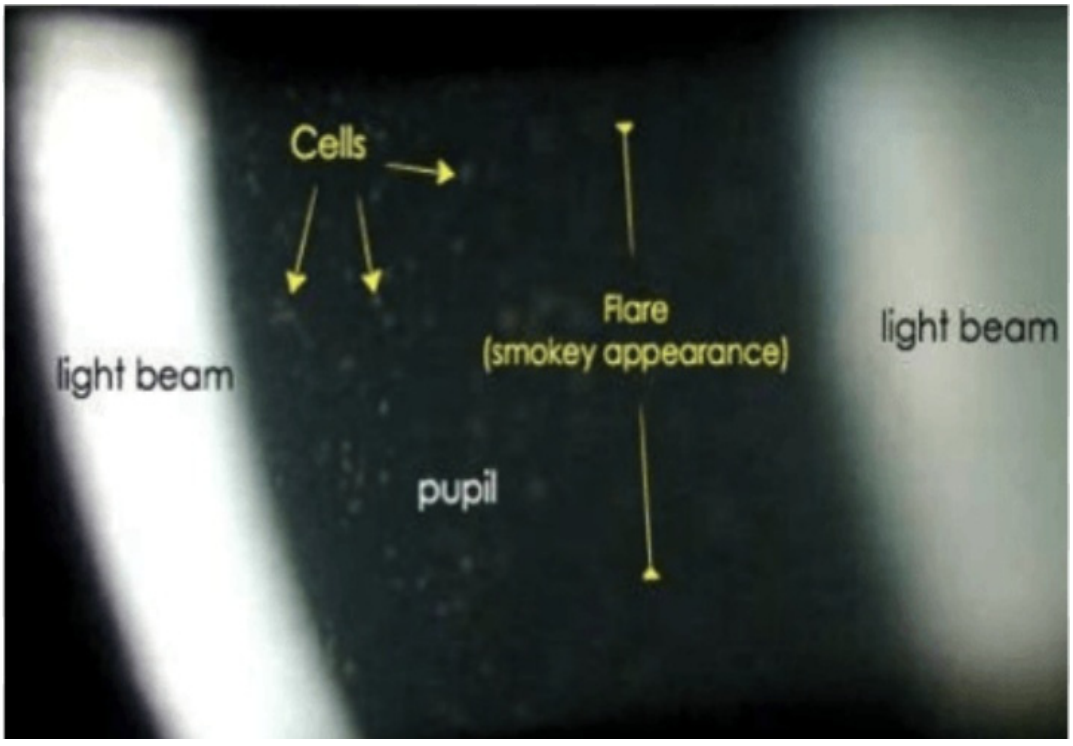
- Flare is a protein escaping from dilated vessels seen as gray or milky in a normally optically empty zone.

- The flare is graded from 0 to 4+ depending upon degree of obstruction of iris[35]. According to Standardization of Uveitis Nomenclature (SUN) Working Group the flares and cells are graded as,

SUN GRADES OF AQUEOUS FLARE

Flare	Grade	Tyndall effect
No flare	0	Nil
Faint	1+	Just detectable
Moderate	2+	Iris details clear
Marked	3+	Iris details hazy
Intense	4+	Severe fibrinous exudates

Figure : 11 **AQUEOUS CELLS AND FLARE**



- Aqueous cells are white blood cells escaping from dilated vessels and seen as white dots which can be counted. Presence of cells in aqueous indicate active inflammations.

SUNGRADES OF AQUEOUS CELLS

Cells	Grades	Tyndall effect
< 5	0	Clear aqueous humour
5 – 10	1+	Aqueous humor nearly clear
11 – 20	2+	Cells definitely identifiable
21 – 50	3+	Hazy iris details
>50	4+	Aqueous humour appears white

5. Hypopyon :

Iritis results in diffusion of toxins. Outpouring of leucocytes from the vessels resulting in cells to gravitate to the bottom of the anterior chamber to form a hypopyon. Hypopyon is absorbed when ulcerative process is controlled.

Figure : 12 **FUNGAL CORNEAL ULCER WITH HYPOPYON**

The slit lamp permits various methods of illumination,

- **Direct illumination:**
  - Direct diffuse
  - Direct focal
    - i) Optical section
    - ii) Conical beam
    - iii) Parallelpiped
- **Indirect illumination:**
  - Indirect focal
  - Retro illumination
  - Sclerotic scatter
  - Specular microscopy

DIRECT ILLUMINATION

Direct diffuse illumination:

The main purpose is to illuminate the eye and its adnexa like lid margins and lacrimal puncta. It is used to define any gross abnormalities.

Applications:

- Sclera examination
- Tear film assessment
- Surface of cornea examination
- General survey of anterior segment
- Staining pattern of ocular tissues in case of corneal ulcer,

Direct focal illumination :

i) Optical section :

In optical section a narrow vertical slit angled to one side of the microscope is used to view the entire cornea. The cornea is scanned from the temporal to nasal limbus.

Application:

- To determine the thickness of cornea and conjunctiva
- To determine the depth or elevation of defect in cornea
- To examine the Bowmans membrane, granular layer of stroma and the inner zone of endothelium.
- To assess the anterior chamber depth

ii) Conical beam :

It is used to assess the anterior chamber for cells and flare by the Tyndall effect its appearance is just like dust floating in the air of sun light filled windows.

iii) Parallelepiped :

In this method the slit width is greater and the height may vary providing three dimensional view of the cornea.

Application:

- Detailed examination of tear film
- Examination of corneal nerves
- Appearance of new blood vessels – diagnosis of chronic or acute inflammation
- Presence of corneal scar
- Presence of corneal striae - lines found in the descemet's membrane and posterior stroma
- Examination of endothelial pigmentation

INDIRECT ILLUMINATION :

Indirect focal illumination:

In this method light enters the cornea through a narrow to medium slit to an area to be examined. It is used to examine infiltrates, corneal scars and epithelial or stromal defects.

Retro- illumination:

It is used to examine corneal vascularization, edema, deposit and folds in the descemet's membrane.

Sclerotic scatter:

The light beam is transmitted through the cornel parenchymal layers. Irregularities of the structure like scars, opacities can be clearly visualized.

VISUAL ACUITY:

'It all begins and ends with vision' is the functional outcome of all structures involved in the eye. Visual Acuity (VA) is defined as the ability to read a standard text pattern at a certain distance. It is expressed in terms of a ratio to normal vision. The visual acuity of normal vision is 6/6 (20/20).

The visual acuity are measured and specified in many ways. The letter chart is the most useful diagnostic and optical modality used to measure visual acuity. The most commonly used chart is Snellen's Chart[36].

SNELLEN'S CHART:

It is the standard eye chart, in use for 150 years.It is named after the Dutch ophthalmologist Hermann Snellen,who developed the chart in 1861,he used abstract symbols.The chart which was published in 1862 used alphanumeric capitals in 5×5 grid and it is the standard chart in use to measure the visual acuity.

Snellen's chart is high contrast chart containing series of letters arranged in lines of diminishing size. The letters used are Sloan letters. Each letter is shaped in such way that it is placed in a square, the sides of which are five

times the breath of the constituent lines and the edges will subtend an angle of 1 minute at the nodal point of the eye. In snellen's chart the largest letter will subtend an angle of 5 minutes at 6m distance from the eye. Thus a person with average visual acuity will be able to read the top letter at 60m , the second line at 36m, the third line at 24m and so on, thus he will be able to read all the letters in the chart at 6m distance. Each line in the snellen's chart is labelled as fractions.

$$\text{Snellen's fraction (VA)} = \frac{\text{Testing distance (meters)}}{\text{Distance at which the size of the letter is read}}$$

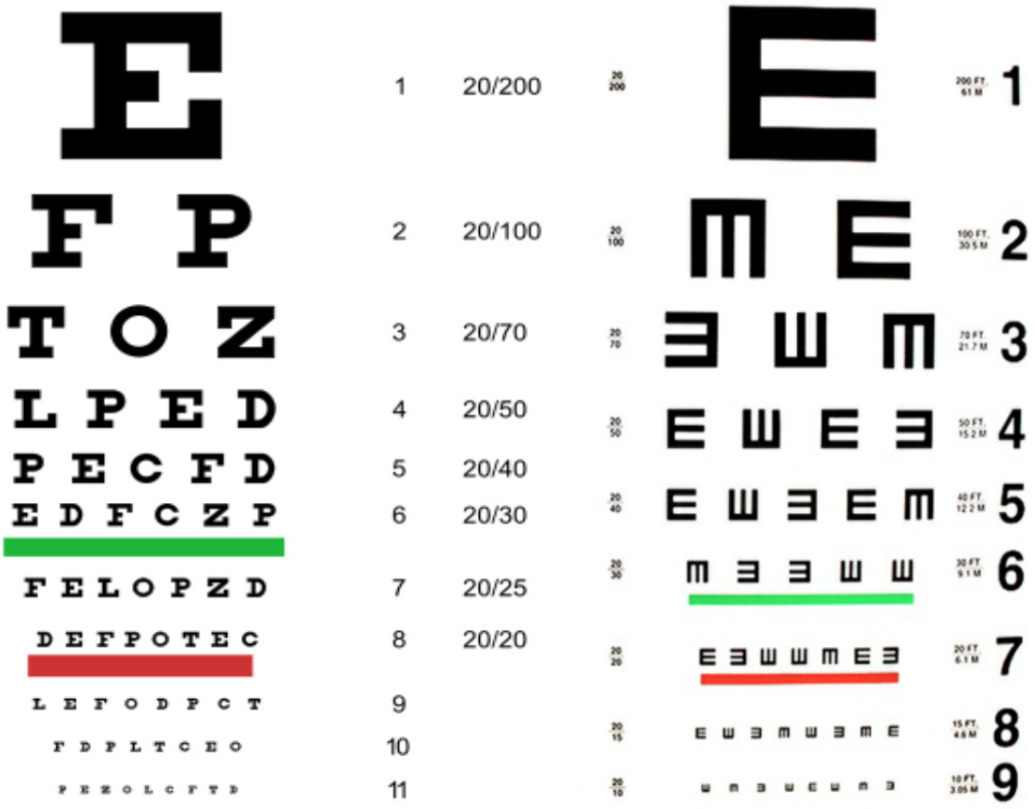
Most of the visual acuity charts are designed to be used at 6m distance and the person with normal vision will be recorded as VA=6/6. But some persons with defective distant vision will be recorded as VA= 6/36, 6/18 and so on depending on the lines he read from 6m distance. But in persons with visual impairment the charts are used at 3m distance and the distant vision recorded will be VA= 3/60. If he is unable to read then the distance is reduced to 2m and the vision is recorded as VA=2/60.

If he is unable to see the top letters at 2m distance he is asked to count the extended fingers held up about 1m and recorded as VA= counting fingers (CFCF) at 1m. If he is unable to count the fingers then the examiners hand is moved in front of the patients eye,if he is able to precise the movements the vision is recorded as VA= Hand movements (HM).

The Latin alphabet chart is useful for the persons who are unable to read. It contains rows of ‘ E’ in various kinds of rotation. Subjects are asked to state where the limbs of E are pointing as up, down, right or left.

In United States of America, this metric system is not usually employed and these values are converted to feet that is 6m= 20 feet, therefore the VA= 6/6 is 20/20 and so on.

FIGURE : 13 **SNELLEN’S AND E- CHART**



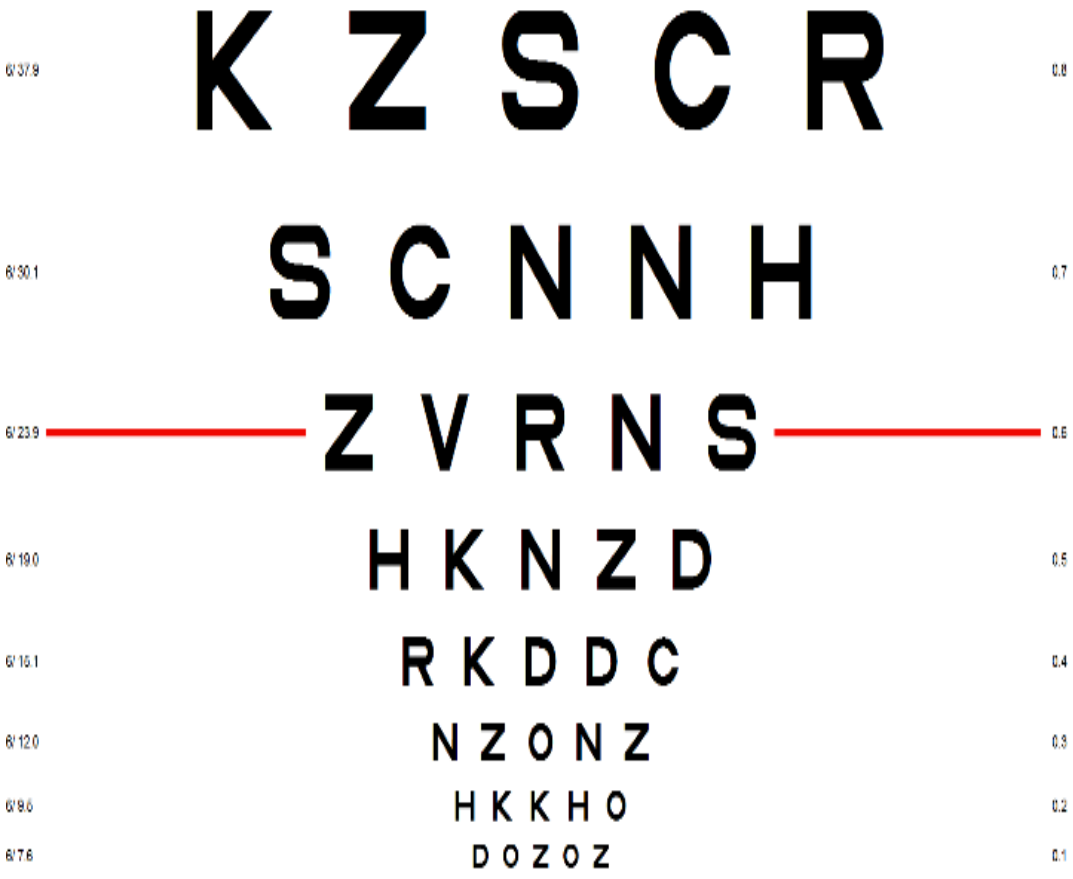
MINIMUM ANGLE OF RESOLUTION:

The drawback with Snellen’s chart is that the denominator value, is the indirect measure of the angle they subtend and size of the letter read. It does not represent equal incremental or decrement in terms of visual difficulty. This is overcome by Log MAR converting the geometric pattern to linear one. The

MAR is arrived at, by dividing the denominator by the distance at which the letters are read ie, Snellen's fraction is inverted or reduced. In log MAR charts each letter read is counted as 0.02 of each line and there are five letters in each line and they are equally spaced and have equal heights and widths.

- ❖ For the vision 6/6 the log MAR value of 0.
- ❖ The acuity becomes worse, when the log MAR value increases.

FIGURE :14 **Log MAR CHART**



SCALE USED FOR MEASUREMENT OF VISUAL ACUITY:

Visual acuity measurement were performed according to the protocol '**Age Related Eye Disease Study using Early Treatment Diabetic Retinopathy Study'** using tumbling E chart and Log MAR visual acuity. The ETDRS chart was introduced in the year 1980s[37].

Table :1 **SNELENS VISUAL ACUITY TABLE**

SNELENS VISUAL ACUITY		**ETDRS VISUAL ACUITY**	
Metric equivalent	**Log MAR Value**	**Metric equivalent**	**Log MAR value**
6/60	1.0	6/60	0.1
6/30	0.7	6/30	0.7
6/20	0.5	6/24	0.6
6/15	0.4	6/19	0.5
6/12	0.3	6/15	0.4
6/10	0.2	6/12	0.3
6/6	0.0	6/6	0.0

According to the criteriathe visual acuity is divided into,

- All vision 0.0-1.0
- High vision 0.0-0.33
- Moderate vision 0.33-0.67
- Low vision 0.67-1.0

PHARMACOTHERAPY:

Although the fungi occur in large family of more than 250,000 species, few fungi species are pathogenic to the eye. Detailed history & clinical examination of the ulcer and conformation of the fungi by laboratory investigations is very important in the initiation of the treatment. The treatment is often prolonged as there is greater difficulty in developing selective antifungal agents than in developing selective antibacterial agents.

Treatment can be started empirically, awaiting laboratory results.

It is divided into,

1. Specific treatment
2. Non-specific treatment
3. Surgical treatment

SPECIFIC TREATMENT:

The specific treatment includes antifungal medications[38].

ROUTES OF OCULAR DRUG ADMINISTRATION:

Routes of drug administration for the treatment of corneal ulcer are chosen with an objective of maximum drug reaching its site of action in sufficient concentration. The various routes are[39]

1. Topical
2. Periocular : Subconjuctival,Sub-Tenon’s, Peribulbar, Retrobulbar
3. Intraocular : Intracameral, Intravitreal
4. Systemic

The most common dosage form is eye drops. The advantages include,

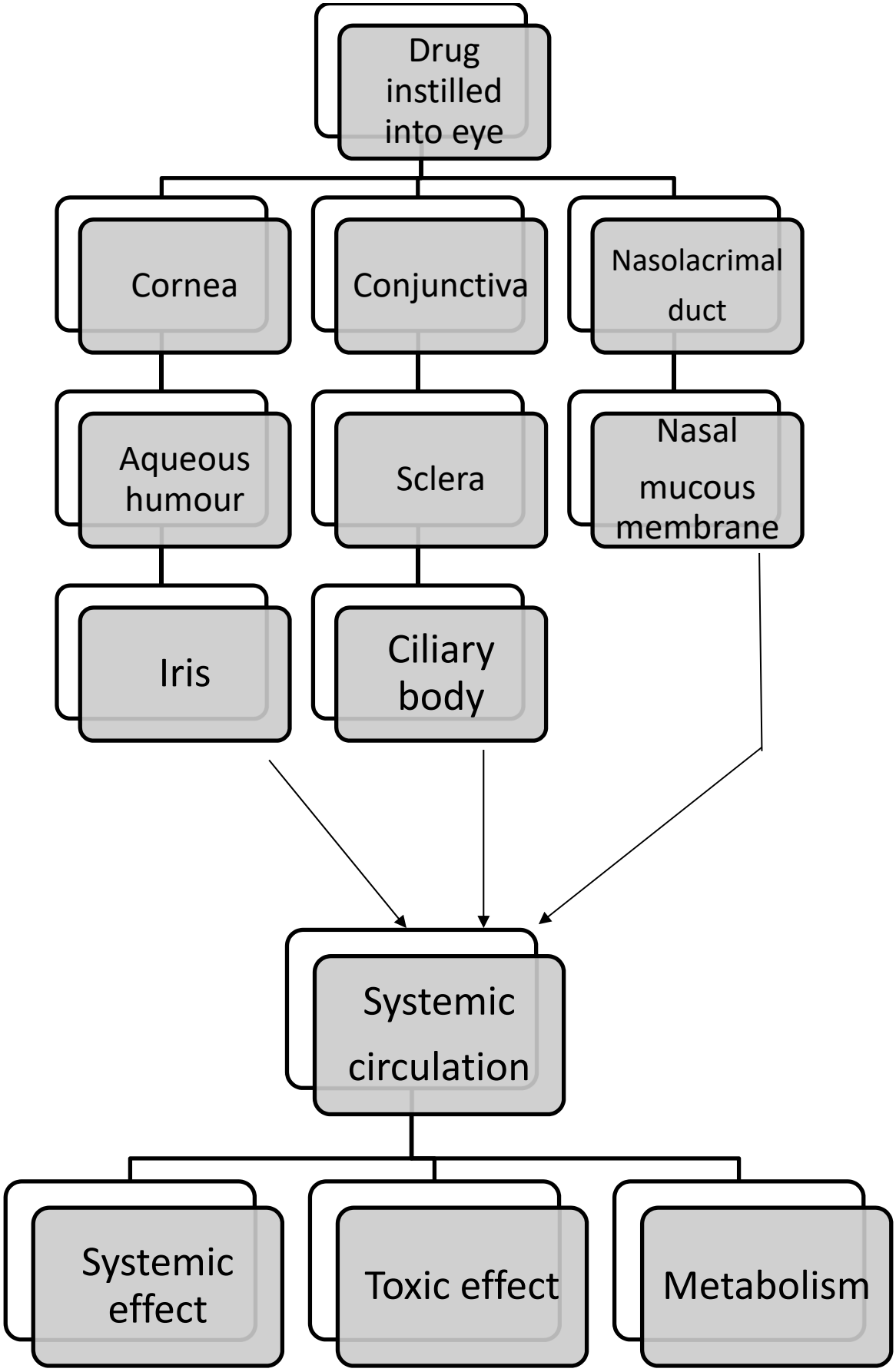
1. It is convenient, simple and painless.
2. The drug effect is localised and a minimum amount of drug reaches the systemic circulation.
3. It also provides higher ocular drug concentration than achieved after systemic administration and has an advantage of avoiding systemic exposure thus systemic adverse effects are minimised.
4. It avoids the hepatic metabolism of the drug.

Drugs in solution and suspension are applied topically on the ocular surface. In order to maximize the therapeutic benefit of the ocular drugs, following method is in practice.

Fraunfelder (1976) described a method, based on tear flow pattern which has the maximum duration of drug retention in the cul-de-sac. Accordingly the head is tilted backwards and the drug is instilled in lower eyelids away from the globe in the cul-de-sac and the patient is asked to look down slowly and to close the eyes gently. The eye is closed for 1minute. Additionally pressure is applied on the medial canthus with closed eyes to prevent the backflow of drug into the lacrimal system.

PHARMACOKINETIC OF OCULAR DRUGS:

On topical application,the drug reaches the systemic circulation mainly through nasolacrimal duct and also through other ocular route[40].


Drug instilled into eye
Cornea
Conjunctiva
Nasolacrimal duct
Aqueous humour
Sclera
Nasal mucous membrane
Iris
Ciliary body
Systemic circulation
Systemic effect
Toxic effect
Metabolism

1) SPECIFIC TREATMENT:

Antifungal medications are[41],

CLASSIFICATION OF ANTIFUNGAL DRUGS :

❖ ECHINOCANDINS Inhibition of fungal cell wall synthesis	❖ Caspofungin ❖ Micafungin ❖ Anidulafungin
❖ POLYENE GROUP Bind to fungal cell membrane ergosterol and increase membrane permeability	❖ Amphotercin B ❖ Natamycin ❖ Nystatin
❖ ALLYLAMINE GROUP Inhibition of ergosterol and lanosterol synthesis.	❖ Terbinafine
❖ AZOLE GROUPS Inhibition of ergosterol synthesis	❖ Ketoconazole ❖ Fluconazole ❖ Itraconazole ❖ Voriconazole ❖ Posaconazole
❖ Inhibition of nucleic acid synthesis	❖ 5- Flucytosine
❖ Distruption of mitotic spindle and inhibition of fungal mitosis	❖ Griseofulvin
❖ Miscellaneous topical agents	❖ Cicloquinol ❖ Naftifine ❖ Butenafine

❖ Topical Azoles	❖ Miconazole ❖ Clotrimazole ❖ Butaconazole ❖ Econazole ❖ Oxiconazole ❖ Sulconazole ❖ Terconazole ❖ Tioconazole

POLYENES:

The polyenes are the oldest group of antifungal agents used in the treatment of fungal infections. The ocular agents commonly in use are,

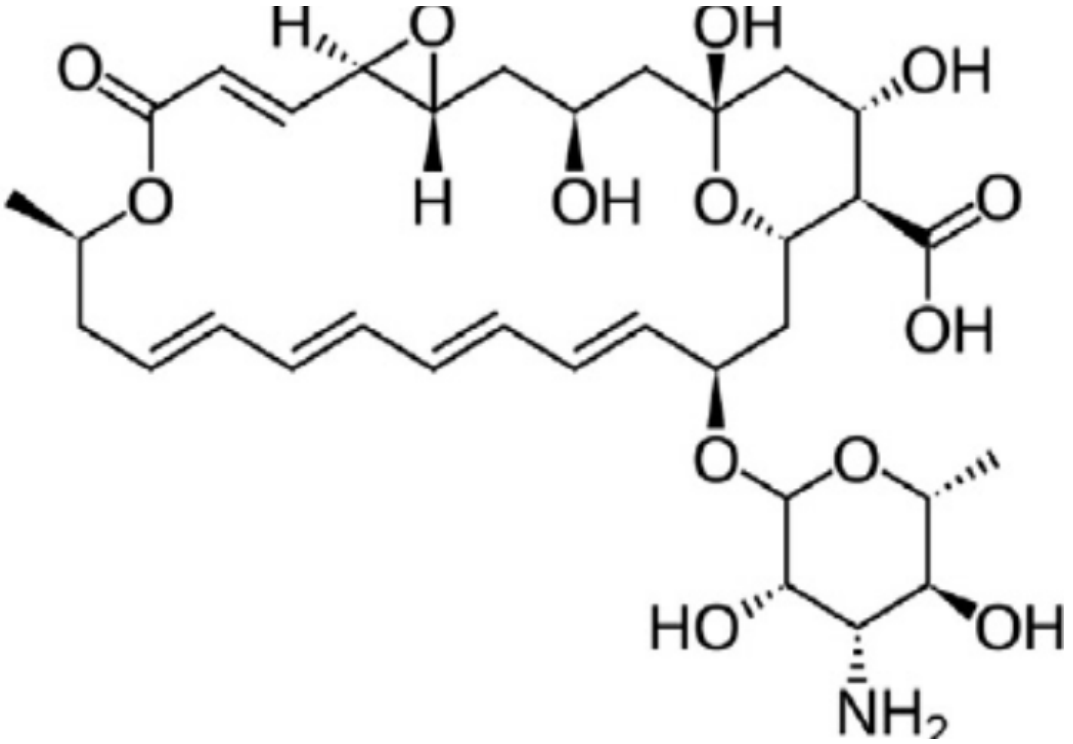
- Amphotercin B
- Natamycin
- Nystatin

NATAMYCIN:

It is the second member of the polyene antifungal group of drugs which was isolated by Struyke et al. in the year 1958 from *Streptomyces natalemsis* species[42].It is the only FDA approved topical ophthalmic antifungal agent in clinical use.

- Molecular formula : $C_{33}H_{47}NO_{13}$
- Molecular weight: 665.7
- Chemical structure : Rich in double bonds

FIGURE: 15 **CHEMICAL STRUCTURE OF NATAMYCIN:**



MECHANISM OF ACTION:

It is a fungicidal drug. Its selective fungicidal effect is because it exploits the difference in lipid composition of fungal and mammalian cell membrane. The cell membrane of fungi contain ergosterol a cell membrane sterol[43]. In human cell and bacterial cell the membrane sterol is Cholesterol.

- Polyenes binds sterols (steroid + alcohol)

- Alters the cell membrane permeability

- By forming pores within the fungal membrane

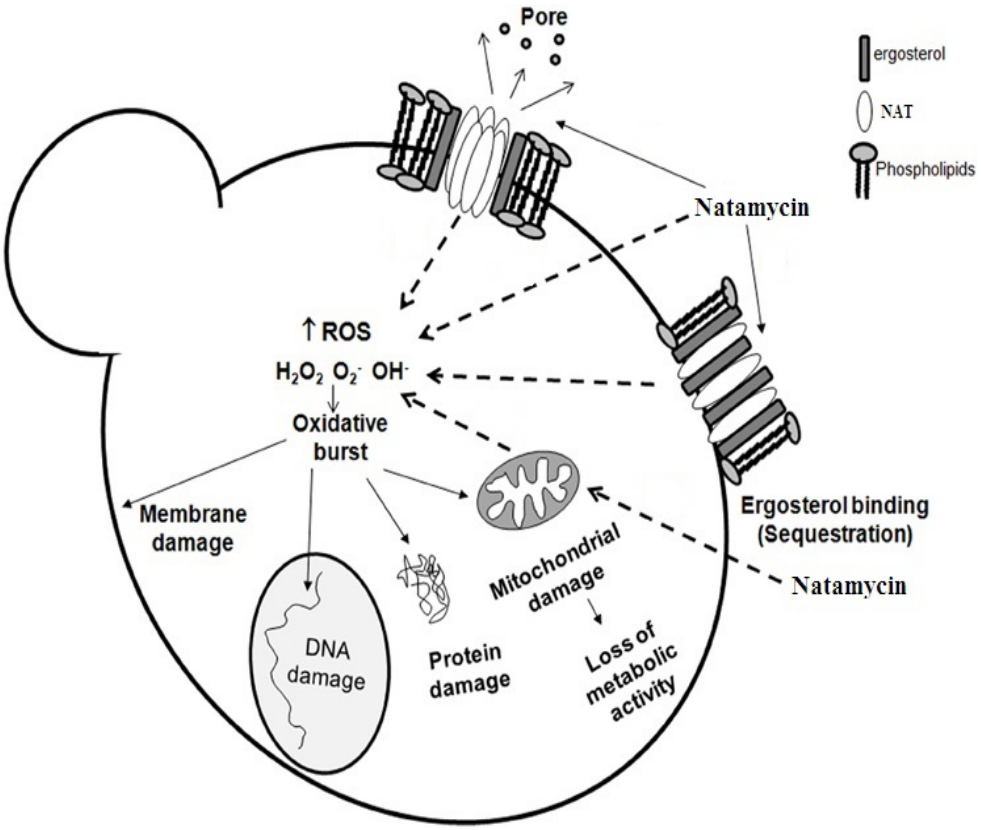
- The polyenes have multiple C= C double bond. Double bond is lipophilic. Hydroxyl rich side is hydrophilic.

- Pores are lined with hydrophilic region lining the inside with the lipophilic portion around the outside of the pore

- Through this pores H^+ and K^+ ions and macromolecules leak out

- Killing the cell

FIGURE: 16 **NATAMYCIN MECHANISM OF ACTION**



RESISTANCE:

- Ergosterol binding is impaired
- Alteration in amount of ergosterol in the membrane[44]
- Modifying the sterol target molecule to reduce its affinity.

CLINICAL PHARMACOLOGY:

- It is the drug of choice against fungal infections with *Fusarium* species.It has an excellent activity against the filamentous fungi including ,
    - *Aspergillus*
    - *Cephalosporium*
    - *Curvularia*

PHARMACEUTICS:

- All polyenes are insoluble in water but stable in 5% suspension.
- It can be stored at room temperature or refrigerated ,but not freezed or exposed to high temperature or light.
- It has poor penetration into deeper structures ,hence effective against superficial infections
- After topical administration it has 2% bioavailability
- The dosing schedules are not yet been established but loading dose of one drop is applied every 30 minutes and increased to hourly for 48 hrs and then for every 2 hrs[45].
- On instillation on to the eye it degrades easily.

PHARMACOKINETICS:

- It is used topically, no systemic absorption.
- It adheres to the cornea so the duration of action is prolonged.
- Though well absorbed from cornea only 2% of the drug is bioactive.
- Minimum Inhibitory concentration of Natamycin is 32 mg/ml

ADVERSE EFFECTS:

The non- serious adverse effects are[46],

- Mild eye irritation
- Redness
- Stinging / burning sensation
- Allergic reactions
- Eye swelling

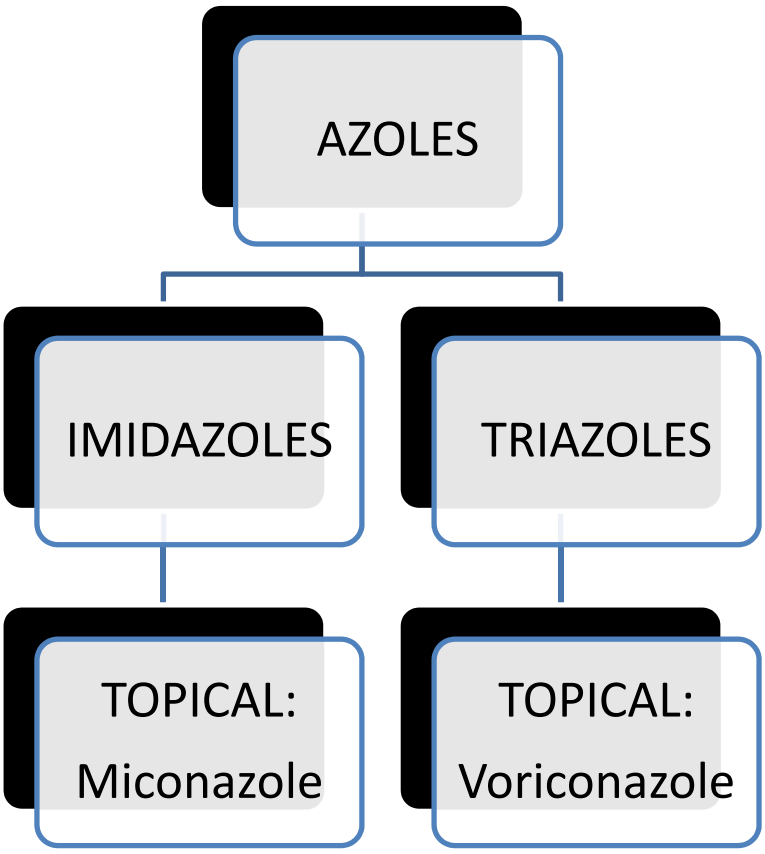
- Eye pain
- Feeling like something in the eye
- Weakness

SERIOUS ADVERSE EFFECTS :

- Change in vision
- Chest pain
- Corneal opacity
- Shortness of breath

AZOLES :

Azoles are synthetic antifungal drugs with broad spectrum of action. Depending on drug concentration it act as fungicidal or fungistatic properties.



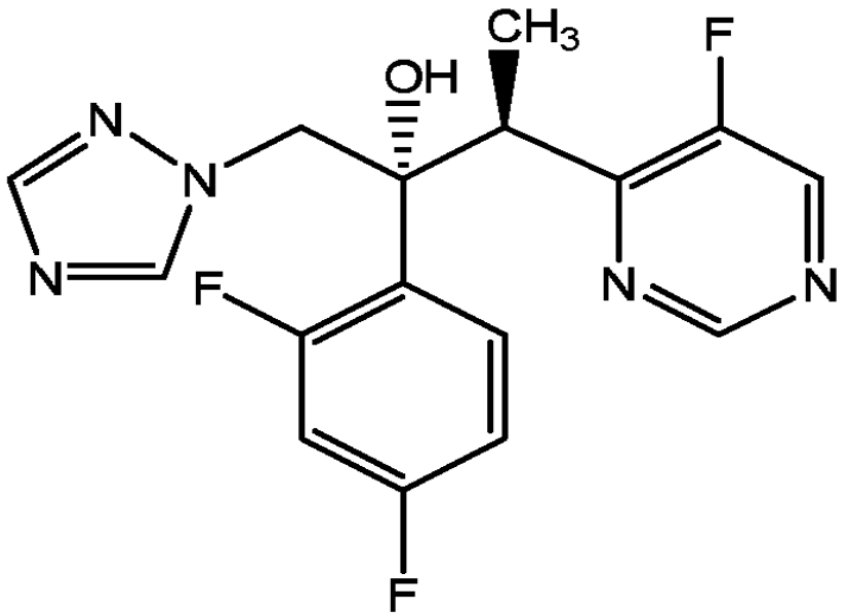
The commonly used azoles in ophthalmology are Clotrimazole, Ketoconazole, Miconazole, Itracanazole, Fluconazole and Voriconazole.

VORICONAZOLE:

It is a second generation synthetic derivative of Fluconazole, and it differs by the addition of a methyl group to the propyl backbone and by substitution of a triazole moiety with a fluropyrimidine group. Voriconazole has been approved by the US Food and Drug Administration for the systemic fungal infection.[47]

- Chemical name: 2,4- difluoro-pyrimidine-4-yl-1-butan-2-ol
- Molecular formula: $C_{10}H_{14}N_5F_3O$
- Molecular weight :349.3

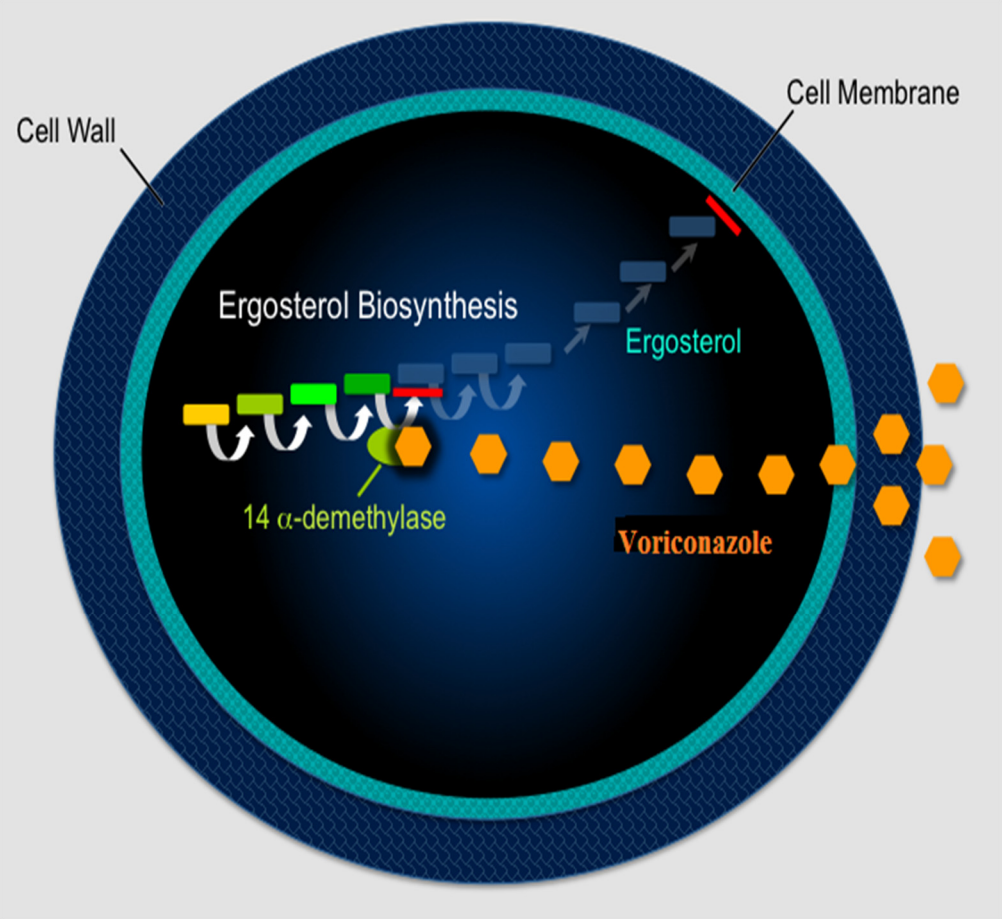
FIGURE :17 **VORICONAZOLE CHEMICAL STRUCTURE:**



MECHANISM OF ACTION :

- The antifungal activity results from reduction of ergosterol synthesis by inhibition of fungal cytochrome P450 enzymes leading to the accumulation of 14-methysterols[48].
- The methysterols disrupt the acyl chains of phospholipids inhibiting growth of fungi by impairing the functions of membrane-bound enzymes.
- They also increase the permeability of the fungal cytoplasmic membrane directly. The effective concentrations are acquired with topical use.

Figure: 18 **MECHANISM OF ACTION OF VORICONAZOLE**



RESISTANCE:

- Alteration of 14 alpha sterol demethylase[44]
- Increased production of 14 alpha sterol demethylase enzyme
- Enzyme efflux

CLINICAL PHARMACOLOGY:

- Azoles are active against *Candida* species,
  - ❖ *Aspergillus,*
  - ❖ *Histoplasmosis,*
  - ❖ *Cryptococcus neoformans*

PHARMACEUTICS:

- Voriconazole is a lipophilic compound with low solubility
- Unstable in aqueous environment[49]
- The eye drop is available with cyclodextrins derivatives,

  (a homologous cyclic oligosaccharides) that increases the solubility, aqueous stability and bioavailability of the drug.
- Voriconazole eye drop is not commercially available[50].It is prepared by diluting the IV formulation of Voriconazole.
- It is available as white lyophilized powder containing 200 mg of Voriconazole and 3200 mg of sulfobutyl ether beta cyclodextrine sodium.
- The powder is reconstituted with 19 ml of water to produce 20 mi of aqueous Voriconazole with a concentration of 10 mg/ml (1%).
- It has an stability for 28 days when prepared as 1% solution.[51]

PHARMACOKINETIC:

- It is available as 1% Voriconazole solution.
- Poorly absorbed from mucous membrane.
- Topical administration of Voriconazole has good penetration through the cornea into the aqueous humour without compromising intra ocular safety[52].
- Minimum Inhibitory concentration of Voriconazole is 0.25-0.5mg/ml
- It has broad spectrum of activity with high intraocular penetration

ADVERSE EFFECTS:

The adverse effects are,

- Sneezing
- Increased sensitivity of eyes to sunlight
- Rashes
- Yellow eye
- Blurred vision
- Swelling of eyes
- Fever
- Itching
- Shortness of breath

GRADING SEVERITY OF ADVERSE EFFECTS:

The adverse events reported are graded into four categories according to the standardized CTCAE **"**Common Terminology Criteria for Adverse Events" (CTCAE,v 3.9)[53].

TABLE : 2 **ADVERSE EFFECTS GRADING**

GRADES:	**SEVERITY:**
GRADE I	**MILD**
GRADE II	**MODERATE**
GRADE III	**SEVERE**
GRADE IV	**LIFE THREATENING**

GRADE I : MILD

- Transient (<48 hrs) or mild discomfort,
- No medical therapy
- No intervention needed

GRADE II: MODERATE

- Mild to moderate limitation of activity
- None but usually minimal intervention/ therapy required

GRADE III: SEVERE

- Marked limitation of activity
- Medical intervention is required
- Hospitalization possible or likely

GRADE IV: LIFE THREATENING

- Extreme limitation of activity
- Immediate emergency medical assistance required to prevent loss of life
- Hospitalization .

KETOCONAZOLE:

It is well absorbed orally when the gastric content is acidic. It is mainly used in the treatment of non-CNS blastomycosis and in silent coccidioidomycosis[54]. It is more effective in treatment of AIDS related oropharyngeal candidiasis. The main adverse effects include gynacomastia, irregular menstrual cycles and elevation of liver enzymes.

FLUCONAZOLE:

Oral absorption is good and does not need acidic pH. It is very effective in the treatment of vulvovaginal, oropharyngeal, mucocutaneous and systemic candidiasis. It is the drug of choice for Candida endophthalimitis. Increased serum transaminase and alopecia are the common adverse effects in patients receiving long term therapy. It has least effect on human cytochrome P450 enzyme.

ITRACONAZOLE:

It is absorbed from GIT at low pH and is more specific for fungal cytochrome P450 enzymes. It is mainly used in the treatment of disseminated or chronic pulmonary histoplasmosis in AIDS patients. It is the drug of choice for cutaneous and extracutaneous sporotrichosis .Higher dose may causes hypokalemia, hypertension and edema. Congestive heart failure has been reported in recent years through pharmacovigilance.

POSACONAZOLE:

Newer triazole licensed in 2006. It has s broard spectrum of action. It is used in *aspergillus i*nfection in severely immune compromised patients. It is well tolerated and inhibits CYP3A4 isoenzymes.

TABLE : 3 **DOSAGE FORMS OF AZOLES**

DRUG	TOPICAL %	ORAL	PARENTRAL
Ketoconazole	1-2%	200-600 mg	
Miconazle	1-2%	200-400 mg	600-1200 mg/d
Econazole	1%		
Clotrimazole	1-2%	60-100 mg	
Fluconazole	1%	100-400 mg	2mg/ml
Itraconazole	1-2%	200-400 mg	

NON-SPECIFIC TREATMENT:

All patients received Non - specific treatment that consist of [13] ,

CYCLOPLEGIC AND ANTI-GLAUCOMA DRUGS:

- Topical 1 % Atropine eye ointment or drops (or)
- Topical 2 % Homatropine eye drops
    - ❖ To reduce pain from ciliary spasm
    - ❖ To prevent the formation of posterior synechiae from secondary iridocyclitis
    - ❖ To increase the blood supply to anterior uvea by relieving pressure on the anterior ciliary arteries

- ❖ Reduces exudation by decreasing hyperaemia and vascular permeability.

- Topical 0.5 % Timolol maleate or oral carbonic anhydrase inhibitor
  - ❖ If intraocular pressure is elevated

SYSTEMIC ANALGESIC:

- Tablet . Paracetamol 500 mg tds (or) Tablet .Ibuprofen 400 mg bd
  - ❖ To relieve pain and to decrease oedema

OTHERS:

- Tablet .Ranitidine 150 mg bd
- Tablet . Vitamins (A,B complex , C)
  - ❖ For early healing of ulcer

PHYSICAL AND GENERAL MEASURES:

- Hot fomentation
  - ❖ To give comfort ,reduces pain and to cause vasodilatation
- Dark goggles
  - ❖ To prevent photophobia
- Rest , good diet & fresh air
  - ❖ To have a soothing effect

SURGICAL TREATMENT:

1) Frequent corneal debridement

Bebulking fungi

Enhance corneal penetration of drugs

2) Therapeutic keratoplasty[55]

3) Other surgical modalities

    - Cyanoacrylate glue
    - Conjunctival flap
    - Amniotic membrane transplantation.

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY CENTER:

The study was carried out in Department of Ophthalmology, Government Rajaji Hospital, Madurai Medical College Madurai.

COLLABORATING DEPARTMENTS:

The study was carried out in collaboration with

- Institute of Pharmacology
- Institute of Microbiology

STUDY DESIGN :

- Single centre
- Open label
- Prospective
- Interventional study

STUDY DURATION :

This study was conducted from December 2015 to June 2017 for a period of 19 months.

1. Literature collection -3 months
2. Designing the study- 1 month
3. Case selection treatment and follow-up 12 months
4. Analysis & Interpretation- 2 months
5. Discussion- 1 month

SAMPLE SIZE :

Total sample size was sixty 60 (30 + 30) cases.

STUDY MATERIALS:

Drugs used were,

Group 1

Topical 5% Natamycin suspension

Group 2

Topical 1% Voriconazole solution

Both the drugs will be applied topically to the cornea every hour during the day while awake and once the infiltrate starts resolving the frequency of the topical drugs is reduced until there is resolution of active ulcer.

ETHICAL APPROVAL:

Institutional ethical clearance was obtained from the Ethical committee, Government Rajaji Hospital, Madurai dated 24-12-2016.

INFORMED CONSENT:

Written informed consent was obtained from all the patients after proper explanation of the study in local language. The consent forms are dated and signed in duplicate by both the patient and the investigator.

SELECTION OF STUDY SUBJECTS:

Newly diagnosed patients of corneal ulcer attending ophthalmology department OPD will be recruited. Corneal scraping will be done for all the patients with corneal ulcer for,

- Gram staining (for identification of infecting organism)
- 10% KOH wet preparation (for the identification of fungal hyphae).

If fungal elements were present they will be selected for the study and the remaining scraping will be sent for culture on Sabouraud's dextrose agar medium for confirmation of fungi (*Fusarium or Aspergillus species*). After satisfying the inclusion and exclusion criteria they will be recruited for the study. The consecutive patients were assigned either to Voriconazole or to Natamycin by Professor, Department of Ophthalmology, Government Rajaji Hospital, Madurai.

INCLUSION CRITERIA:

- Presence of non severe corneal ulcer at presentation that satisfy Jone's protocol

Severity of keratitis based on definition by Jone's protocol[56]

TABLE : 4 **JONE'S PROTOCOL**

NON-SEVERE	SEVERE
6mm diameter - ulcer	>6mm diameter
Ulceration involving superficial one third of the cornea	Ulceration involving deep one third of the cornea
Suppuration of the superficial two third of the corneal layer	Suppuration involving the deep one third of cornea

- Age between 25-50 years
- Evidence of filamentous fungus on smear (potassium hydroxide wet mount, Gram stain)
- The patient must be able to verbalize

Subjects willing for the study were explained about the proposed study and the need for follow up. Only those subjects who accepted to adhere to the guidelines were included for the study.

EXCLUSION CRITERIA :

The patient who had any one of the following or a combination of them were excluded,

- Aetiology of keratitis other than fungal
- Impending perforation or descemetocele, corneal perforation
- Immunodeficiency, autoimmune disorder, neurotrophic lesion , dry eye and chronic dacryocystitis.
- Known allergy to study medication (antifungal /preservative)
- Best spectacle corrected vision worse than 6/60 (20/200) in fellow eye
- Pregnancy & lactating mothers
- Patients not willing to participate

DISCONTINUATION CRITERIA:

Patients were permitted to discontinue at any time during the study. And when the patient was found to develop another illness or worsening of existing illness or requiring additional drugs they will be withdrawn from the study .

CASE SELECTION:

Sixty cases were selected over a period of one and half year. Counseling was given to them about the proposed study. Patients were informed verbally and in writing by the investigator about the nature, significance, implications and risks of the study prior to enrolment in language

and in terms that were easy to understand by the patient. The details of the investigator (name, mobile number and contact address) were given to each and every patient to enable them to contact for any ailments at any time during the entire study period.

- Patients were assigned into two groups, each group comprising 30 patients after satisfying inclusion and exclusion criteria.
- A detailed history of patients are recorded. A thorough clinical examination was conducted for all patients.

The character of the ulcer will be assessed using a slit lamp microscopy such as,

- Epithelial defect
- Infiltrate size (mm around the ulcer)
- Depth of ulcer (% of stroma involved)
- Satellite lesion (present or absent)
- Hypopyon (present or absent)

Patients were screened for relevant investigations before subjecting them into the study.

INVESTIGATIONS:

OPHTHALMIC EXAMINATION:

VISUAL ACUITY (VA):

- Best spectacle corrected visual acuity in both eyes is evaluated before and after initiation of therapy.

- The visual acuity is evaluated by using Snellen's /Tumbling E chart and Log MAR (Minimum angle of resolution) value is derived by conversion table.
- Visual acuity outcome were performed according to a protocol adapted from the **Age Related Eye Disease Study (AREDS 1999) using Early Treatment Diabetic Retinopathy Study** as best vision, counting fingers, hand movement, light perception and no light perception.
- After initiation of treatment, vision is tested at the end of the therapy (3 month) and is compared with the initial vision.

RELAPSE:

- All participants will be followed for the next 3 months for recurrence of the ulcer if any.

DUCT:

- Patency of duct in both the eyes is tested before the start of the treatment.

ADVERSE DRUG REACTIONS:

- Adverse drug reactions as given below will be monitored, during the course of antifungal medications,

BASIC INVESTIGATIONS:

- Blood sugar
- Renal function test
- Urine routine

STUDY MONITORING, AUDITING AND INSPECTION:

In order to ensure adherence to guidelines, audit and inspections were done by Ophthalmology Department and Pharmacology Department.

STATISTICAL ANALYSIS:

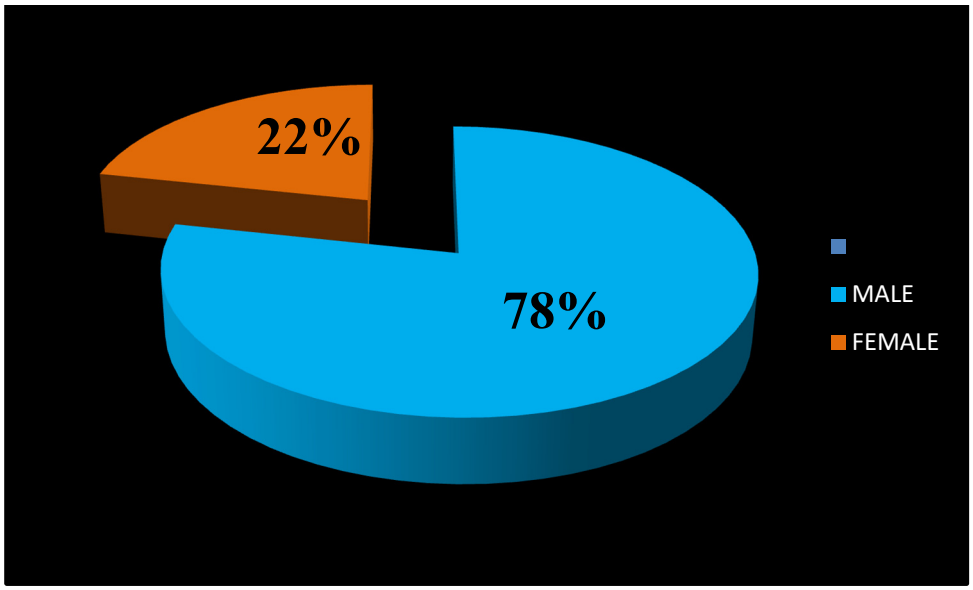
The data were analyzed with SPSS statistical software package (Version 16.0 SPSS Inc., Chicago, USA). The time taken for healing of corneal ulcer and the visual acuity will be measured and the efficacy and adverse effects will be monitored between two groups by using unpaired t test, and $P<0.05$ will be considered as statistically significant.

RESULTS

RESULTS

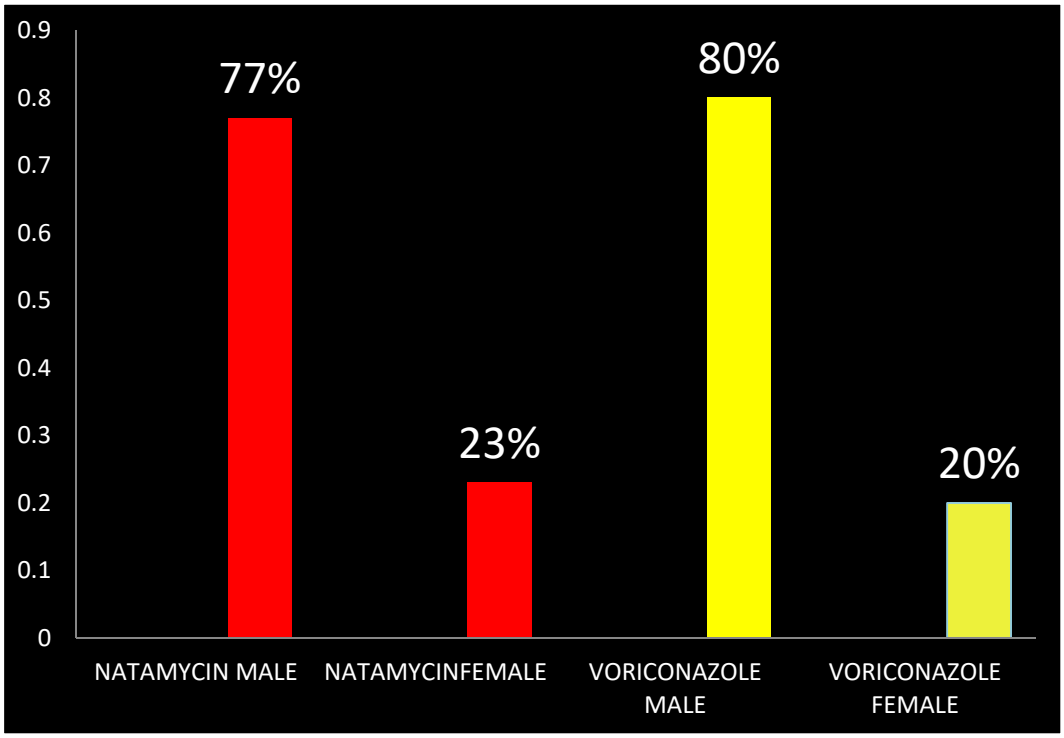
The study was conducted in the Department of Ophthalmology to study the efficacy and tolerability of topical Natamycin and Voriconazole in treatment of filamentous fungal keratitis. Sixty patients were recruited for the study after satisfying the inclusion and exclusion criteria. The patients were divided into two groups, Group I received topical 5% Natamycin suspension and group 2 received topical 1% Voriconazole solution for 3 months and the cure ratio is assessed for 3 months using different parameters and observed for the next 3months for relapse.

Figure-:19 **GENDER DISTRIBUTION - MALE –FEMALE**



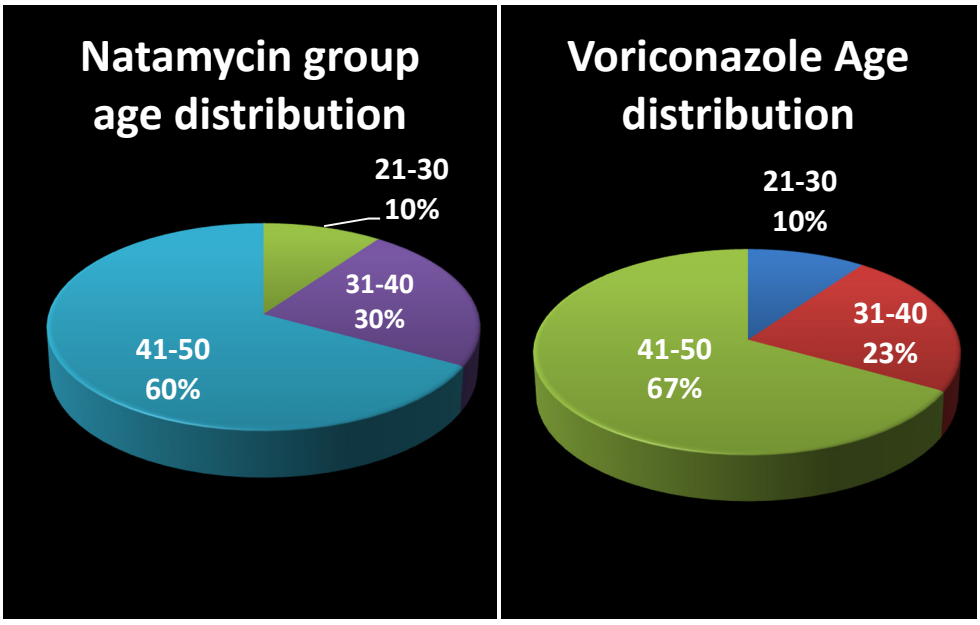
All the patients completed the trial and there was no drop out in the study. All the 60 patients were analyzed for the response to the drug therapy and tolerability.

Figure:20 **SEX DISTRIBUTION**



Among sixty patients analyzed (78%) were males and rest were females (22%). Group I had (77%) males and (23 %) females, while the Group II had (80 %) males and (20 %) were females.

Figure:21 **AGE DISTRIBUTION**



The age distribution of Group I had the following distribution with 3(10%) patients belonged to 21-30 years, 9 (30%) were between 31-40 years, 18 (60%) belonged to 41-50 years.

The age related distribution of Group II was as follows, 3(10 %) patients were in the age group of 21-30 years ,7 (23 %) were in 31-40 year and 20 (67%) were in 41-50 years.

***TABLE : 5* AGE DISTRIBUTION**

AGE GROUP YEARS	NATAMYCIN GROUP I (n =30)	VORICONAZOLE GROUP II (n =30)	TOTAL NO OF PATIENTS (n =60)
21-30	3 (10%)	3(10%)	6(10%)
31-40	9(30%)	7(23%)	16(27%)
41-50	18(60%)	20(67%)	38(63%)
TOTAL	30	30	60

MODE OF INJURY:

The most common risk factor for fungal keratitis is trauma. The initiating trauma is mainly due to vegetable matters or organic matters. About 19 persons in group I and 21 in group II the mode of injury is by vegetable matter and about 2 in group I and 5 in group II the main cause of injury is by metals. Other modes of injury that include injury with unknown objects was about 9 in group I and 4 in group II.

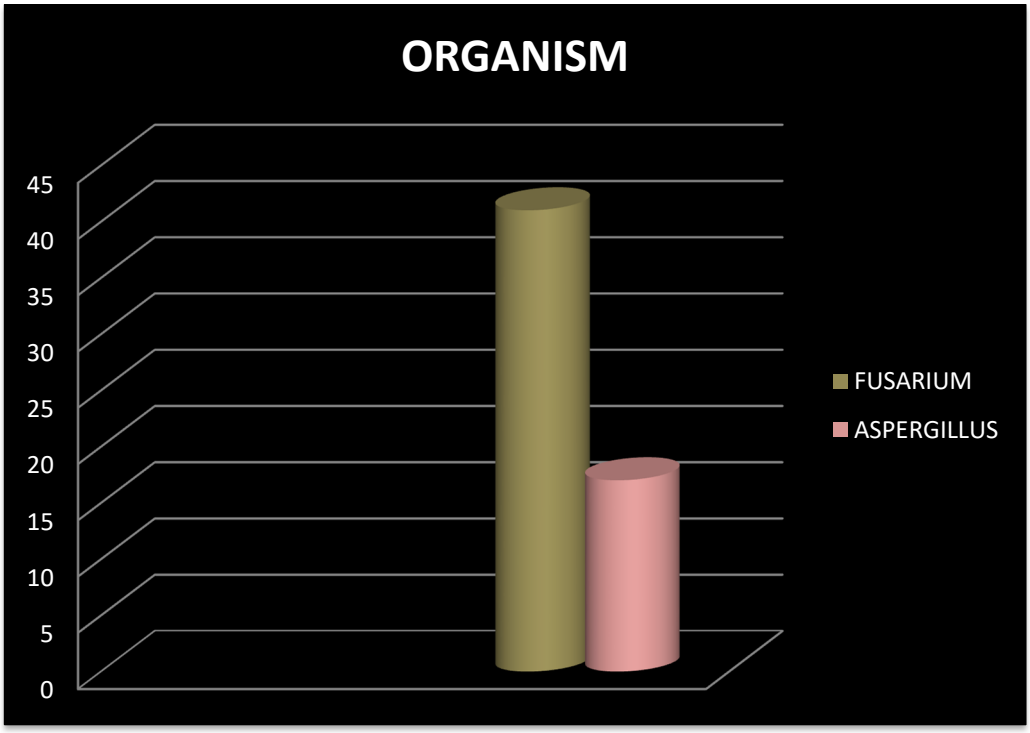
TABLE : 6 **BASELINE DEMOGRAPHIC CHARACTERISTIC**

TRAUMA /INJURY NO.			
CHARACTERISTIC	**NATAMYCIN (n =30)**	**VORICONAZOLE (n =30)**	**TOTAL (n = 60)**
Vegetative matter / wood	**19**	**21**	**40**
Metal	**2**	**5**	**7**
Unknown object	**9**	**4**	**13**
Contact lens	**0**	**0**	**0**

MICROBIOLOGY:

Fungal identification was performed using microscopic and cultural characteristic by corneal scraping. The incidence of occurrence of *fusarium* species in Natamycin treated group was 70%, total *Aspergillus* species was 27%, out of which *fumigatus* was 7% *, flavus* was 17% and *terreus* was 3%.

Figure:22 **MICRO ORGANISM**



In case of Voriconazole treated patients *fusarium* species accounts for 67% and *aspergillus* species was 30% of which *fumigatus* accounts for 10% and *flavus* 20%.Other species incidence was 3% in both Natamycin and Voriconazole group.

TABLE : 7 ORGANISM

DRUGS	**ORGANISMS**					
Species	**Fusarium**	**Aspergillus 8(27%)**				**Others**
		A.flavus	**A.fumigatus**	**A,niger**	**A.terreus**	
NATAMYCIN	**21(70%)**	**5(17%)**	**2(7%)**	**0(0%)**	**1(3%)**	**1(3%)**
VORICONAZOLE	**20(67%)**	**6(20%)**	**3(10%)**	**0(0%)**	**0(0%)**	**1(3%)**

Thus *Fusarium* is the most common organism in causing fungal keratitis in all age group.

EFFICACY :

The efficacy of the topical antifungals was assessed by visual outcome at the end of 3rd month by using a BSCV (Best Spectacle Corrected Vision) and LogMAR values using ARED score (Age Related Eye Disease Study) from the baseline to the end of the study, that is at the end of 3rd month and the results observed for both the groups are as follows,

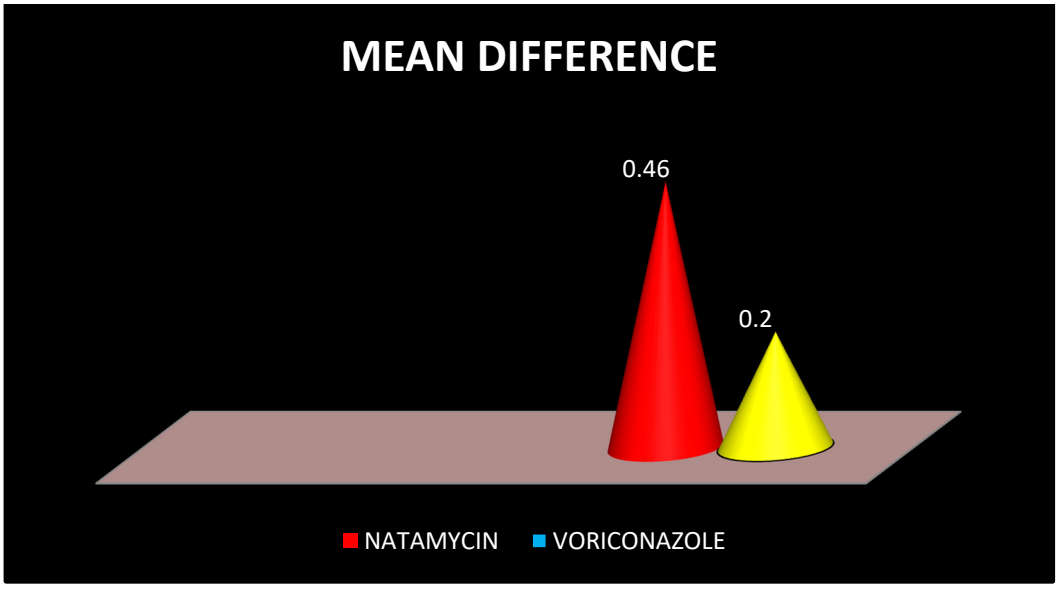
TABLE: 8 **COMPARISION OF EFFICACY BETWEEN TWO GROUPS**

DRUG GROUP	Log MAR BASELINE	Log MAR 3 MONTH	MEAN DIFFERENCE	P VALUE
NATAMYCIN GROUP	1.09±0.25	0.63±0.26	0.46	0.00 (<0.05)
VORICONAZOLE GROUP	1.31±0.29	1.11±0.41	0.20	0.016 (<0.05)

In Natamycin group, the mean Log MAR score at baseline was 1.09 ± 0.25 and at the end of 3 month was 0.63 ± 0.26.The mean difference was 0.46. In Voriconazole group, the mean Log MAR score at baseline was 1.31 ± 0.29 and at the end of 3 month was 1.11 ± 0.41. The mean difference was 0.20.

The efficacy of two antifungals was compared using paired students t test and the results were statistically significant ($p< 0.00$) imparting that there was a difference between the two groups in visual acuity from baseline score to the end of the study.

Figure:23 **MEAN DIFFERENCE**



According to the ARED score, the visual outcome of Natamycin group falls in moderate vision [0.33-0.67] group and for Voriconazole group ,falls in the low vision [0.67-1.00]. So Natamycin seems to have better efficacy in visual outcome compared to Voriconazole in the treatment of filamentous fungal keratitis.

RESPONSE RATE:

The parameters observed are epithelial defect, infiltrate size and depth of the ulcer. The response rates for the two antifungals was assessed by observing the healing rate of epithelial defect to scar formation , reduction in infiltrate size & depth of the ulcer and also by the time of onset of resolution.

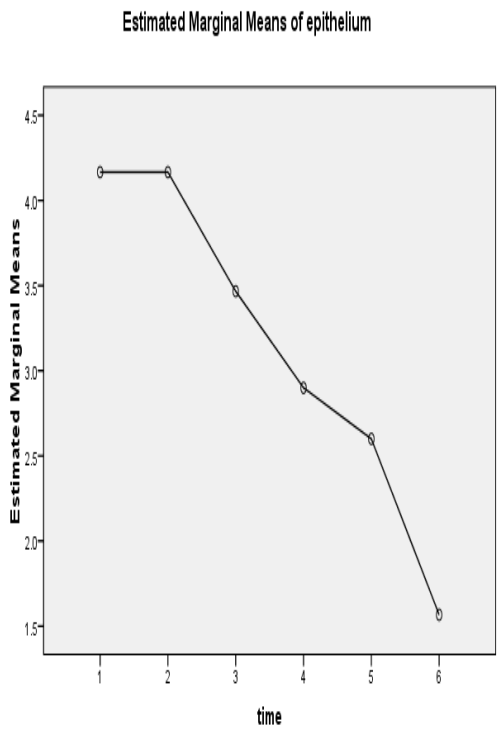
EPITHELIAL DEFECT:

In Natamycin group the baseline epithelial defect was found to be 4.17 ± 0.98 and reduction in the epithelial defect was observed at 3rd week from beginning of the treatment and the mean epithelial defect after treatment was reduced to 2.90± 0.96. Multiple Anova and post hoc Bonferroni test was applied, and it was found that the reduction in epithelial defect is highly significant (p<0.00) from third week of treatment.

Figure:24 **EPITHELIAL DEFECT**

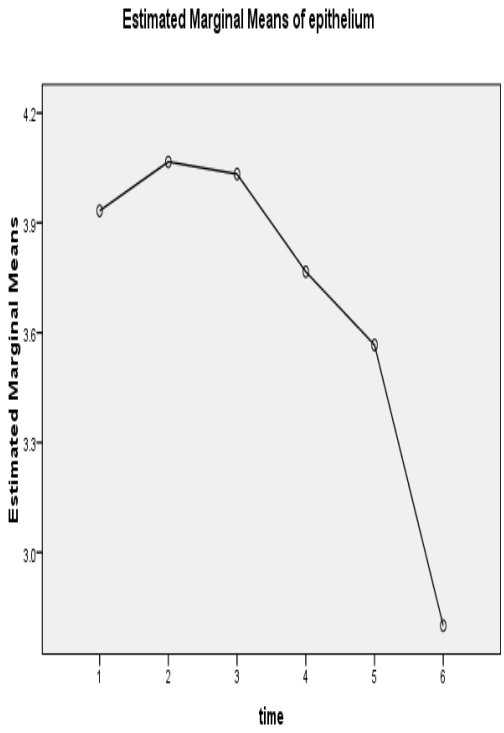
NATAMYCIN

f value :102.38



VORICONAZOLE

f value:14.87



In Voriconazole group the baseline epithelial defect was found to be 3.93± 1.17 and reduction in the epithelial defect was observed at 5th week from beginning of the treatment and the mean epithelial defect after treatment was reduced to 2.80± 1.42. Multiple Anova and post hoc Bonferroni test was applied, it was found that the reduction in epithelial defect is significant ($p<0.01$) from fifth week of treatment.

SCAR:

At the end of the treatment the scar was measured depending on the intensity of the light passing through the scar and the scar was categorized as nebula , macula and leucoma. The distributions of the scar in two groups are,

TABLE : 9 **SCAR TYPE**

DRUG GROUP	SCAR TYPE		
	NEBULA	MACULA	LEUCOMA
NATAMYCIN	21	8	1
VORICONAZOLE	12	10	6

INFILTRATE SIZE:

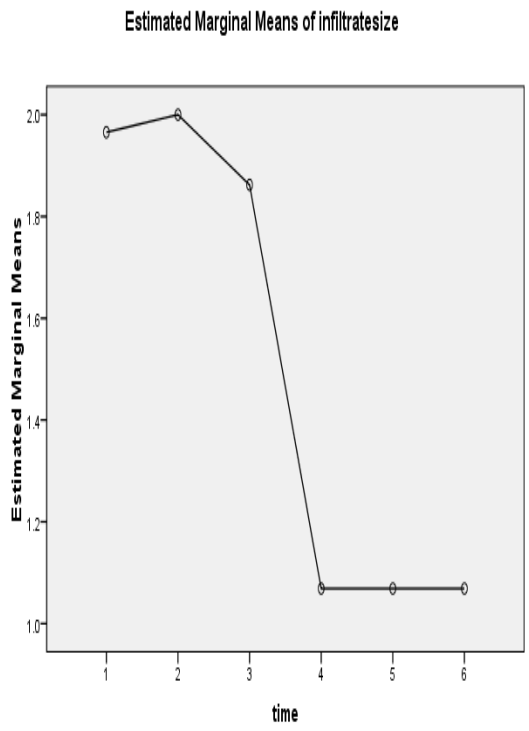
In Natamycin group the baseline infiltrate size was found to be 1.97 ± 0.18 and reduction in infiltrate size was observed at 3rd week from beginning of the treatment and the mean infiltrate size after treatment was reduced to 1.07± 0.371. Multiple Anova and post hoc Bonferroni test was

applied, it was found that the reduction in infiltrate size is highly significant ($p<0.00$) from third week of treatment.

Figure:25 **INFILTRATE SIZE**

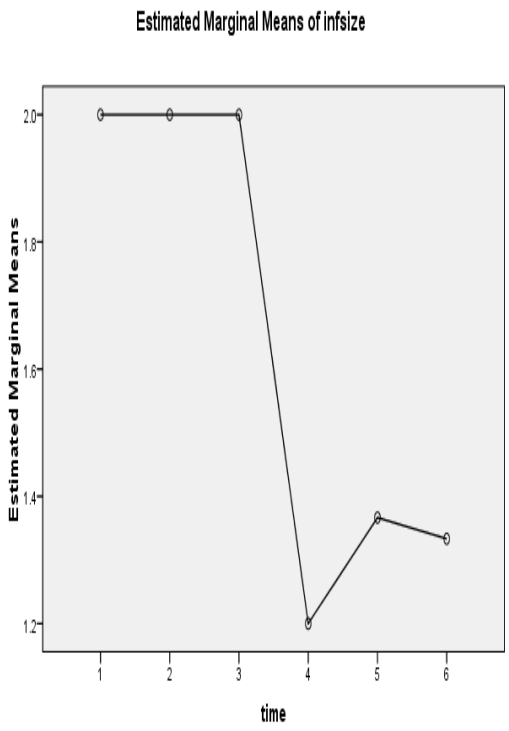
NATAMYCIN

f value: 102.24



VORICONAZOLE

f value: 24.20



In Voriconazole group the baseline infiltrate size was found to be 2.00 ± 0.00 and reduction in infiltrate size was observed at 5thweek from beginning of the treatment and the mean infiltrate size after treatment was reduced to 1.33 ± 0.758. Multiple Anova and post hoc Bonferroni test was

applied, it was found that the reduction in infiltrate size is significant (p<0.00) from fifth week of treatment.

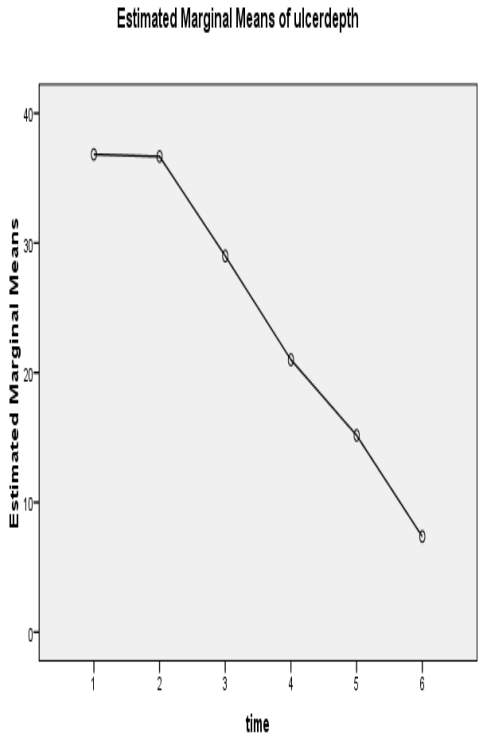
ULCER DEPTH:

In Natamycin group the baseline ulcer depth was found to be 36.83 ± 10.544 and reduction in ulcer depth was observed at 4th week from beginning of the treatment and the mean depth after treatment was reduced to 15.17± 12.35. Multiple Anova and post hoc Bonferroni test was applied, it was found that the reduction in ulcer depth is significant (p<0.00) from fourth week of treatment.

Figure :26 **ULCER DEPTH**

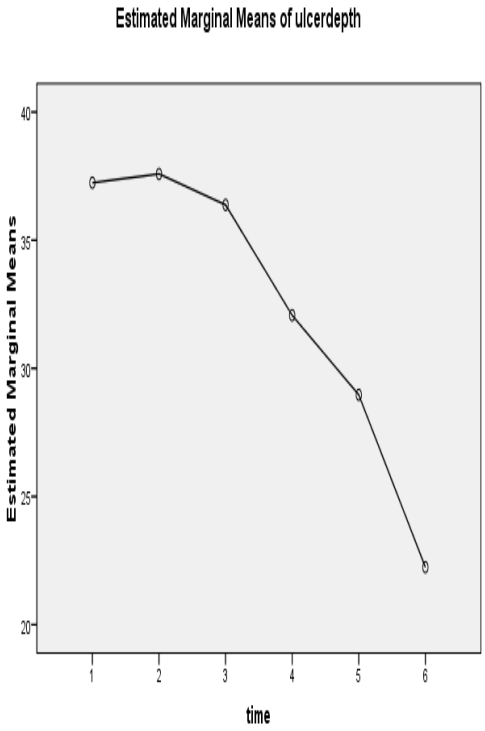
NATAMYCIN

f value: 132.29



VORICONAZOLE

f value: 31.14

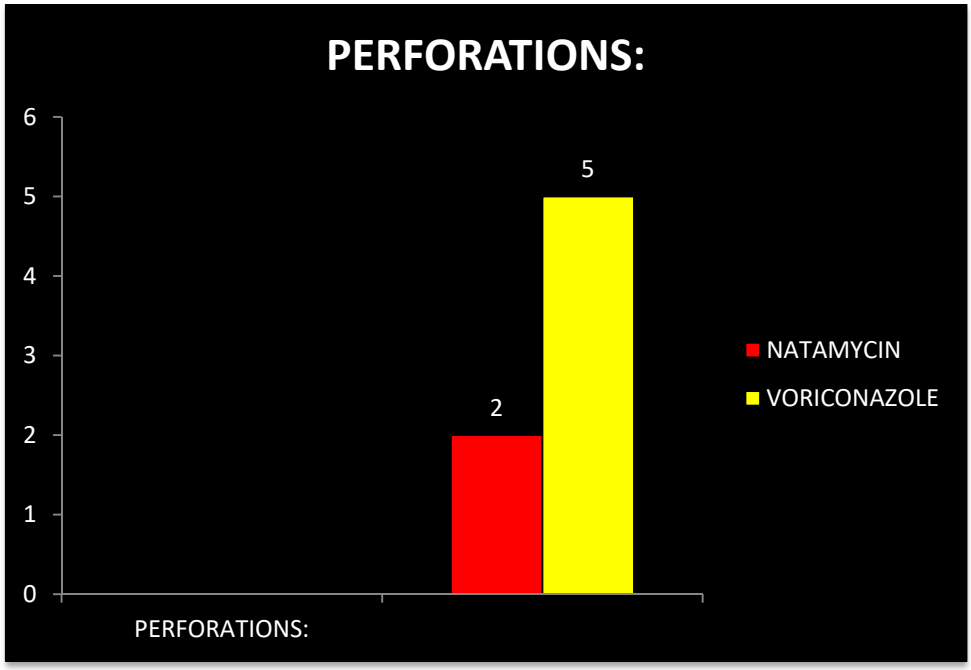


In Voriconazole group the baseline ulcer depth was found to be 37.24± 8.720 and reduction in ulcer depth was observed at 5thweek from beginning of the treatment and the mean depth after treatment was reduced to 22.24± 16.23. Multiple Anova and post hoc Bonferroni test was applied, it was found that the reduction in ulcer depth is significant ($p<0.00$) from fifth week of treatment.

PERFORATION:

The perforation in Natamycin group was two and in Voriconazole group it was five during the treatment.

Figure:27 **PERFORATION**



TOLERABILITY:

The tolerability of the two topical antifungals was evaluated by counting the number of adverse effects reported by the patients, according to the standardized CTCAE "Common Terminology Criteria for Adverse Events" (CTCAE,v 3.9). Accordingly the tolerability was graded as ,Grade I: mild, Grade II : moderate, Grade III: severe and Grade IV: life threatening.

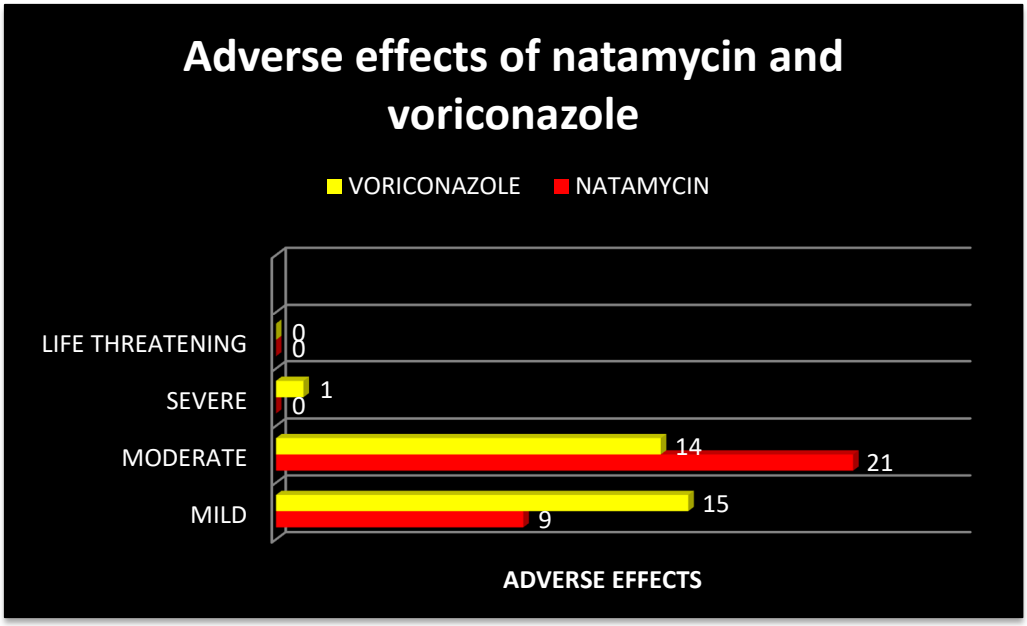
TABLE :10 **COMPARESION OF ADVERSE EFFECTS**

DRUG GROUP	MILD	MODERATE	SEVERE	LIFE THREATENING
NATAMYCIN GROUP	9(30%)	21(70%)	0	0
VORICONAZOLE GROUP	15(50%)	14(47%)	1(3%)	0

In Natamycin group 9(30%) had mild adverse events,21(70%) had moderate event and no one had sever and life threatening events.

In Voriconazole group15(50%) had mild reaction,14(47%) had moderate effects,1(3%) had severe reactions and no one had life threatening adverse effects.

Figure:28 ADVERSE EFFECTS

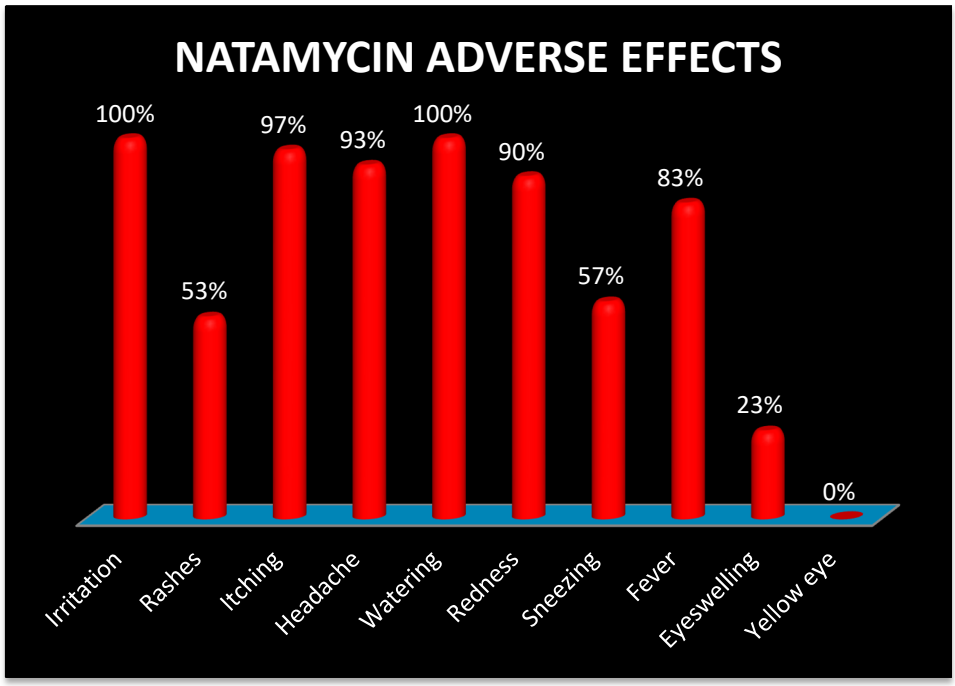


SAFETY:

The safety profile was evaluated by comparing the adverse effects reported by patients in both groups to standard adverse effect check list prepared for the study.

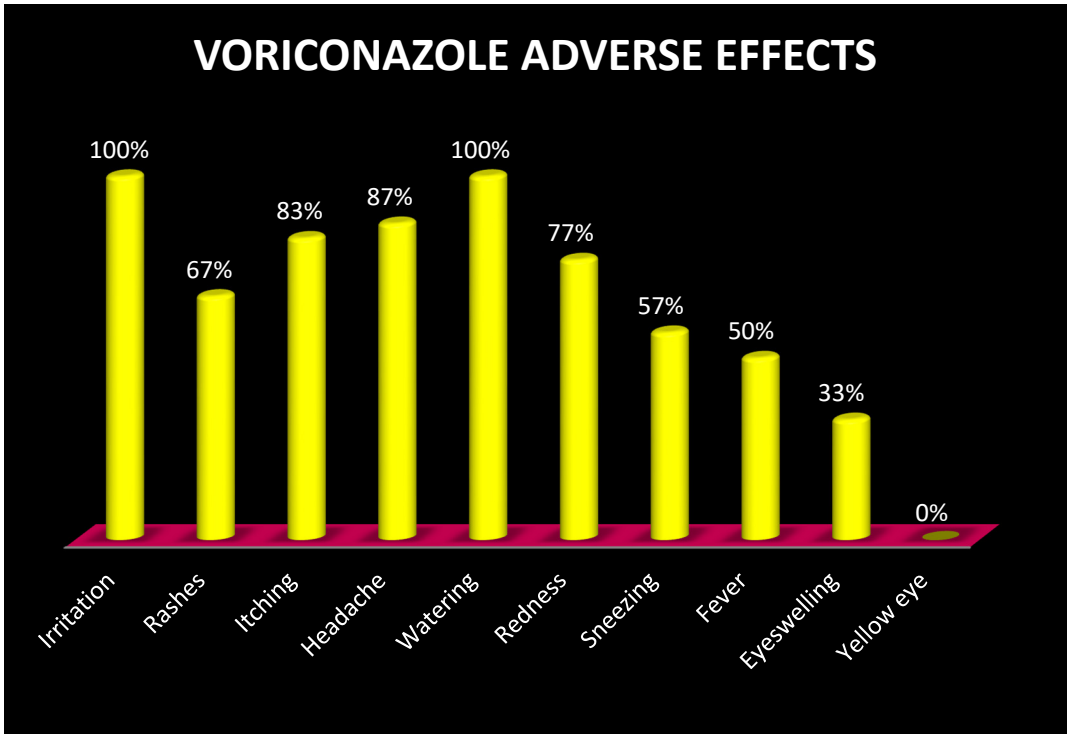
The adverse effects shown by Natamycin group is 100% for irritation & watering, 97% for itching, 93% for headache, 90% for for redness, 83% for fever, 57% for sneezing, 53% for rashes, 23% for eye swelling and 0% for yellow eye.

Figure:29 **ADVERSE EFFECTS OF NATAMYCIN**



The adverse effects shown by Voriconazole group is 100% for irritation & watering, 87% for headache, 83% for itching, 77% for redness, 76% for rashes, 57% for sneezing, 50% for fever, 33% for eye swelling and 0% for yellow eye.

Figure:30 **ADVERSE EFFECTS OF VORICONAZOLE**



BIOCHEMICAL PARAMETER:

Biochemical parameters were normal for all the participants before starting the study and the same biochemical parameters were repeated at the end of the study. The parameters were normal for all the participants there was no alteration in renal function and blood sugar by both the topical antifungals.

TABLE: 11 BIOCHEMICAL PARAMETERS

BIOCHEMICAL PARAMETERS	MONTHS	NATAMYCIN	VORICONAZOLE
BLOOD SUGAR	INITIAL	93.1 ± 10.02	92.1 ± 9.9
	3 MONTHS	90.1 ± 9.4	91.2 ± 9.7
BLOOD UREA	INITIAL	30.7 ± 6.6	28.7 ± 5.7
	3 MONTHS	31.5 ± 5.1	27.5 ± 5.6
SERUM CREATINNINE	INITIAL	0.58 ± 0.1	064 ± 1.02
	3 MONTHS	0.62 ± 0.14	0.68 ± 0.2
URINE ALBUMIN	INITIAL	NIL	NIL
	3 MONTHS	NIL	NIL

DISCUSSION

DISCUSSION

Mycotic keratitis (International Nomenclature of Disease number 2100) is a general term for a mycosis of the cornea, synonyms include 'keratomycosis' and 'oculomycosis' which is caused by wide variety of fungi.[57] It is potentially a life threatening ocular infection. Keratitis is usually manifested as severe inflammation, followed by ulcer and hypopyon formation. It is diagnosed by the presence of fungal hyphae within corneal stroma.

Introduction of new class of antifungals has significantly changed the management of corneal ulcer. But early identification and treatment at the grass root level that is, at the primary care level may be beneficial to the patient in reducing the suffering. It is also beneficial to the community in bringing down the mortality and morbidity due to vision impairment or blindness caused by fungal corneal ulcer.The pharmacological management of ulcer has seen many advances with the advent of newer drugs and different modes of delivery of drugs.

The molecular biology of ulcer has been studied in detail with the advent of newer techniques like confocal microscopy[58], polymerase chain reaction and genotyping. The disease pathology learnt in depth and has focused newer targets of drug action.

These continuous efforts of many researchers globally, has given us many pharmacological tools to treat the fungal corneal ulcer. Drugs with better efficacy and tolerability are preferred among the older and newer antifungals.

Natamycin one of the oldest antifungal in use, is a tetraene polyene that has been regarded as an important agent in treatment of fungal keratitis. Its efficacy was well established in the treatment of fungal corneal ulcer.

Voriconazole a newer triazole group of antifungal claimed to be equally effective as polyenes in treating corneal ulcer with better tolerability and with lesser side effects.

In the present study, treatment of filamentous fungal corneal ulcer with topical Natamycin and Voriconazole was compared and evaluated in a tertiary care hospital present in southern region of Tamil Nadu, in order to prevent the blindness in younger population and to initiate the earliest possible treatment.

Efficacy of visual acuity was assessed at the end of three month, using ARES (Age Related Eye Study) score, tolerability and safety were assessed using the standardized **"**Common Terminology Criteria for Adverse Events" (CTCAE,v 3.9) severity grading scale. The data obtained was subjected to statistical analysis and yielded the following results.

Among sixty patients analyzed, the incidence of fungal keratitis is more in males. The most common mode of injury is trauma with vegetable matter.

In this present study, visual outcome was studied between two groups. The visual outcome in Group I (Natamycin) falls in moderate vision group [0.33-0.67] and in group II (Voriconazole) falls in the low vision [0.67-1.00] as per AREDS score. Thus Natamycin group ($p<0.00$) has better visual outcome than with Voriconazole group with statistical significance.

For the treatment of filamentous fungal corneal ulcer Natamycin or Voriconazole is the first line regimen. Three randomised control studies have compared Natamycin and Vorionazole topical eye drops in treatment of superficial filamentous fungal corneal ulcer. All these studies shown that Natamycin treated patients was associated with significant visual outcome compared with Voriconazole treated patients.

According to the randomized study by Prajna *et al*, the visual outcome was better with Natamycin (0.18 log MAR) than with Voriconazole.[59]

In a study conducted by Sharma S et al, Natamycin was more effective in the treatment of fungal keratitis than Voriconazole in visual outcome.[60]

In temperate countries like India, filamentous fungi have been reported as causative agent of fungal corneal ulcer. In the present study,100% of culture positive cases were filamentous organisms. The most common isolates being *Fusarium* and *Aspergillus* species. Natamycin has been reported as the most effective medication in treating these organisms.

According to the study conducted by P. Lalitha et al, Natamycin had good activity against both *Fusarium* and *Aspergillus* species [61].

In antifungal susceptibility of fungal keratitis conducted by Suman et al and Orapin et al in eastern India ,they showed that *fusarium* species was most sensitive to Natamycin and *Aspergillus* to Voriconazole. [62,63]

In the present study the response to Voriconazole was better for patients with *Aspergillus* infections when compare to the response shown by the patients on Natamycin treatment.

Fungal keratitis usually responds slowly to antifungal medications over a period of weeks. Clinical signs of improvement include decrease in size of infiltrate, disappearance of satellite lesion, reduction in hypopyon size and healing of epithelial defect to scar formation.

The healing of epithelial defect was observed from 3rd week from the beginning of the treatment in Natamycin group and from 5th week in Voriconazole group. Complete healing in both the groups highlights that the penetration of both the drugs through the cornea is effective with topical route itself. Penetration of Voriconazole through the intact corneal epithelium have been proven effectively. [64]

In a recent study conducted by Ishank Gupta et al the epithelial defect and depth of the ulcer resolved earlier by fourth week in Natamycin treated group than Voriconazole treated group.[65]

Infiltrate size reduction was observed from 3rd week in natamycin group and 5th week in voriconazole group. Infiltrate is measured in millimetre around the ulcer. Patients with dense infiltrate more than its size, resulting in thick scar (leucoma) . Thick scar will not permit the light to pass through it will result in poor visual outcome.

In a study conducted by Shahzad I Main et al, size of the infiltrate becomes well demarcated initially followed by reduction in size. The scar size

mainly depends on the size of the infiltrate. Denser the infiltrate more will be the scar size[66].

Ulcer depth indicates the percentage of stroma involved. Reduction of ulcer depth was observed from 4th week in Natamycin group and 5th week in Voriconazole group. Greater the ulcer depth the risk of perforation is more. The perforation is marginally more with Voriconazole compared with Natamycin.

In the present study, non severe corneal ulcer was selected for both the groups (Natamycin Vs Voricnazole). Normally corneal epithelium is lipophilic and stroma is hydrophilic, and hence stroma has a barrier effect on lipophilic drugs. Voriconazole being lipophilic when given to patients with dense infiltrates, its stromal penetration is reduced. Hence healing is suppressed which may even go for perforation.[67]

Older the age with large infiltrate size, large epithelial defect and large involvement of stroma (ulcer depth) at presentation significantly predicted a longer time of re-epithelialization and poor visual outcome in both groups.

The systemic adverse effects of both the antifungals were not studied in detail in previous studies. The present study have elaborated the adverse effects of both the drugs. In Natamycin group adverse effect was mild in 30%, moderate in 70% and in Voriconazole group mild was 50%, moderate was 47% and severe was 3%.

In a study conducted by Arora et al, there was no significant adverse effects noted with Natamycin and minimal systemic effects with Voriconazole[68].

Though Voriconazole at 1% solution is stable for 14 weeks at 2-8◦C has to be maintained at pH of 7 it is associated with severe adverse effects like skin rashes but needs no intervention. Natamycin though having a moderate adverse effects needs intervention. [69]

Natamycin is available as 5% suspension for topical administration, is more economical and cost effective when compared with 1%topical Voriconazole costs higher and is not commercially available.

Hence from this study, the efficacy and tolerability of topical 5% Natamycin and 1% Voriconazole studied in our centre and the study objectives have been achieved. The efficacy of both the antifungals in the treatment of non severe fungal keratitis patients were studied in detail which shows that Natamycin is superior to Voriconazole in visual outcome and clinical cure of ulcer but considering the tolerability, Voriconazole is better than Natamycin and it is statistically significant.

SUMMARY

AND

CONCLUSION

SUMMARY AND CONCLUSION

Fungal keratitis is an important disease of public concern that accounts for a substantial load of corneal blindness in a country like India, mainly dependent on agricultural income. The prevalence of fungal keratitis is more in males than in females.

Microbiological investigation is essential for correct diagnosis and treatment of fungl keratitis. Filamentous fungi are the most frequently reported pathogens. Fungal corneal ulcer is more difficult to treat than bacterial corneal ulcer with worse outcome. Early and rapid diagnosis and specific drug therapy can prevent mono ocular vision loss and mortality.

To treat the mycotic keratitis effectively, a drug must be non toxic to the eye, must penetrate the eye well and have a high level of antifungal activity against at least one significant ocular pathogen. Antifungal agents that are useful in the treatment of fungal keratitis include topical 5% Natamycin , 0.15-0.3% Amphotercin B, 1% Clotrimazole, 1% Miconazole, 1% Voriconazole.

Topical 5% Natamycin is usually chosen as initial therapy for the treatment of fungal keratitis. Voriconazole is a new generation triazole antifungal with broad spectrum of activity and high ocular penetration. It has been reported to be useful in the treatment of fungal keratitis.

Based on this view, the present study was undertaken to study the efficacy and tolerability of the Natamycin and Voriconazole in the treatment of filamentous fungal keratitis. The visual outcome at the end of the study was

higher and fall in moderate vision group in Natamycin treated group as per the Age Related Eye Disease Study protocol compared with Voriconazole which falls in poor vision. But the visual acuity of Voriconzole was better for the patients with *Aspergillus* infection than with *Fusarium* species.

The healing rate of the ulcer like epithelial defect, resolution of ulcer depth and reduction of infiltrate size starts by 3rd to 4th week in Natamycin treated group and in Voriconazole group the resolution starts from 4th to 5th week after the start of the treatment.

Tolerability of Voriconazole is better than Natamycin, but the perforation is greater with Voriconazole treated group in comparison with Natamycin group.

Since this is an interventional study with small sample size of 30 patients per group, dose relationship could not be explained, for the development of adverse effects in both the groups. So, further studies are needed to confirm the tolerability of the both the drugs.

From this study, it is evidenced that Natamycin is cost effective and superior than Voriconazole in visual acuity outcome, but the tolerability was better with Voriconazole compared with Natamycin. However the incidence of corneal perforation was higher with Voriconazole group than with Natamycin group.

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PROFORMA

Name

OP/IP No.

Age

Sex

Occupation

Address

Socio economic status

Phone no.

H/o presenting illness :

Past history

Family h/o:

Occupational H/o:

Nature of work:

Menstrual history:

Personnel History:

Dietary Habits.

Treatment History:

Drug History

H/O about drug compliance:

H/O any adverse Effect to drugs:

General examination

O/E

Conscious

Oriented

VITALS	**OTHER SYSTEMS**
Pulse:	Central Venous System
Blood Pressure	Respiratory System
Respiratory Rate :	Per abdomen
Temperature	Central nervous system

OCULAR EXAMINATION:

PARAMETER	RIGHT EYE	LEFT EYE
1.Duct		
2.Vision		

- Assessment of clinical characteristics of ulcer such as,

  Epithelial defect

  Infiltrate

  Depth of ulcer

  Hypopyon size

  Satellite lesion

- Assessment of tolerability, best spectacle-corrected visual acuity (BSCVA) & Log MAR value.

ADVERSE REACTIONS	NATAMYCIN	VORICONAZOLE
Irritation		
Itching		
Watering		
Blurred vision		
Redness		
Sneezing		
Yellow eye		
Headache		
Dry mouth		
Rash		

Base line investigations

PARAMETERS	**VALUES**
1.Blood urea	
2.Serum creatinine	
3.Blood sugar	
4.Urine routine	

PATIENT INFORMATION SHEET

PATIENT INFORMATION SHEET

Who can be contacted for further questions?

For further questions regarding this clinical study or your rights as patient and participant in the study, please contact your doctor who will always be ready to provide you the necessary information.

If you have experienced any health related problems as well as in case of hospitalization please contact your doctor.

Name and Address of the Contact Person:

_____

_____

Phone number: _____

Please take a copy of this information sheet home with you.

INFORMED CONSENT FORM IN ENGLISH

CONSENT FORM IN ENGLISH

Full name of the patient(in capital letters): _____

Address:_____

_____Date of Birth:_____

Patient no:_____ Sex :_____ I freely agree to participate in the above – mentioned clinical study.

My doctor_____ informed me in a personal counseling interview about the study drug, possible side effects and risks, the nature, objective and significance of this clinical study and my responsibilities resulting thereof. In addition, I read and understood the contents of the Patient Information Sheet and Informed Consent Form. The doctor answered all questions in an adequate and comprehensible manner. I had sufficient time to decide on my participation in this clinical study.

I will follow the instruction of my doctor, which are essential for the performance of this clinical study. I have the right to withdraw from the study at any time without giving any reason and without any disadvantage for me.I confirm that I have not participated in this study and I have not taken part in another study within the last 30 days prior to the start of the study.

I received one original of the Patient Information Sheet together with the signed Information Consent Form.

_____

(Place, Date & Signature of the Patient)

_____

(Place, Date & Signature of the Doctor)

INFORMED CONSENT FORM IN TAMIL

நோயாளியின் பெயர்: _____ வயது:_____ இனம்:_____

விலாசம்: _____

தகவல் அளிக்கப்பட்டஒப்புதல் படிவம்

மேற்குறிப்பிட்டமருத்துவஆய்வில் ஓர் பங்கேற்பாளராகசேர்க்கப்பட்ட இதன் மூலம் நான் சுதந்திரமாகஎன் ஒப்புதலைஅளிக்கிறேன்.

ஆய்வு மருந்து பற்றி ஒரு தனிப்பட்டஆலோசனைநேர்முகவிளக்கத்தில் என் ஆய்வுமருத்துவவர் ஆய்வுமருந்து, சாத்தியமாகும் விளைவுகள் மற்றும் அபாயங்கள், இயல்பு, இந்தமருத்துவ ஆய்வின் நோக்கம் மற்றும் முக்கியத்துவம் பற்றிமற்றும் அதனால் ஏற்படும் எனதுபொறுப்புகள் பற்றி எனக்குதகவல் தெரிவிக்கின்றார். இதோடு கூடுதலாக, நான் தேதியிட்ட எனக்கு அளிக்கப்பட்ட நோயாளிக்கான தகவல் தாள் மற்றும் தகவல் அளிக்கப்பட்ட ஒப்புதல் படிவத்தில் அடங்கியவிபரங்கள் பற்றிபடித்து புரிந்து கொண்டுள்ளேன். மருத்துவர் போதிய மற்றும் விரிவான விதத்தில் என் பங்கேற்பு பற்றித் தீர்மானிக்க எனக்குப் போதிய நேரம் இருந்தது.

இந்த மருத்துவ ஆய்வு நடத்தப்பட்ட மிக முக்கிமானதாக என் மருத்துவரின் குறிப்புகளை நான் பின்பற்றுவேன். எந்தகாரணமும் அளிக்காமல், எனக்குஎந்த நஷ்டமும் ஏற்படாமல் எந்த நேரத்திலும் ஆய்வை விட்டு விலக எனக்கு உரிமை உண்டு.

இந்தமருத்துவஆய்வில் சேகரிக்கப்படும் எனது சொந்த தகவல், குறிப்பாக எனது மருத்துவ ரெகார்டுகளில் எனது பெயர் மற்றும் பாலினம் மற்றும் இனம் குறிக்கப்படும் என்பதற்கு நான் சம்மதிக்கிறேன் இந்த தகவல் ஆனது

1. எலக்ட்ரானிகல் முறையில் அல்லது ஒருபகுதி காகிதவடிவில் பதிவு செய்யப்படும் பத்திரமாக வைக்கப்படும் மற்றும் மதிப்பீடு செய்யப்படும்.

2. விஞ்ஞான மதிப்பீடு மற்றும் கூடுதல் விஞ்ஞான உபயோகத்திற்காக மற்றும் அளிக்கப்படும்.

3. உகந்ததேசியமற்றும் சர்வதேச ரெகுலேட்டரி அதாரிட்டிகளுக்கு அனுப்பப்படும்.

இதோடு மட்டுமின்றி அங்கீகரிக்கப்பட்ட பிரதிநிதிகள் எனது சொந்த விபரங்கள் உடனான மருத்துவ ரெகார்டுகளை பரிசோதிக்கலாம். விஞ்ஞான மதிப்பீடு மற்றும் மருத்துவஆய்வின் செயல் திறனுக்காக தகவலை முழுமையாக சரியாகப் பரிமாற்றம் செய்ய இது உதவுகிறது.

நான் இந்தஆய்வில் இதுவரைபங்கேற்று இருக்கவில்லைமற்றும் இந்த ஆய்வு ஆரம்பிக்கும் முன்பு 30 நாட்களில் நான் மற்றொரு ஆய்வில் பங்கேற்றிருக்கவில்லை என்பதை உறுதிசெய்கிறேன்.

நோயாளிக்கானதகவல் தாளின் ஒருஅசல் உடன் கையெழுத்ததிடதகவல் அளிக்கப்பட்டஒப்புதல் படிவத்தைநான் பெற்றுள்ளேன்.

நோயாளி:

_____ _____ _____

பெயர் பெரியஎழுத்துகளில் கையெழுத்து தேதி சாட்சி:

_____ _____ _____

பெயர் பெரியஎழுத்துகளில் கையெழுத்து

தேதி

நோயாளிக்கு உறவுமுறை:

நான் டாக்டர் மேற்கண்ட

பெயருடைய நோயாளிக்கு

நோக்கம் மற்றும் தன்மை பற்றி விளக்கியுள்ளேன்

ஆய்வின்

என்பதை உறுதி செய்கிறேன். மேலும் நான்

அனைத்து ஆய்வு சம்பந்தப்பட்ட கேள்விகளுக்கும் பதில்கள்

மற்றும்

அளித்துள்ளேன்.

ஆய்வின் நிபந்தனைகளைஅவர்களுக்கு விளக்கியுள்ளேன்

என்பதை உறுதி செய்கிறேன்.

மருத்துவர்:

பெயர் பெரிய எழுத்துகளில் கையெழுத்து தேதி

MASTER CHART

NATAMYCIN MASTER CHART													
S.No	**Age**	**Gender**	**Vision**	**Epithelial Defect**	**Infiltrate size**	**Ulcer depth**	**Hypo pyon**	**Satellite Lesion**	**End of first month**	**End of second month**	**End of third month**	**Vision**	**Relapse**
1	47	Male	1.8	4mm	UTMS	25%	Present	Present	Decreased	Decreased	Nebula	0.47	No
2	36	Female	1.3	3mm	UTMS	20%	Present	Present	Decreased	Decreased	Nebula	1	No
3	41	Male	1	3mm	UTMS	35%	Present	Absent	Decreased	Decreased	Nebula	0.47	No
4	48	Male	1.8	5mm	UTMS	50%	Present	Present	Decreased	Decreased	Macula	0.6	No
5	27	Male	1	4mm	UTMS	40%	Present	Absent	Decreased	Decreased	Nebula	0.77	No
6	47	Female	1.3	5mm	UTMS	45%	Present	Present	Decreased	Decreased	Macula	0.6	No
7	44	Male	1	5mm	UTMS	50%	Present	Present	Increased	Increased	Leucoma	1.8	No
8	35	Male	1.3	4mm	UTMS	30%	Present	Present	Decreased	Decreased	Nebula	0.77	No
9	38	Male	1	4mm	UTMS	35%	Present	Present	Decreased	Decreased	Nebula	0.47	No
10	42	Male	1.1	3mm	UTMS	30%	Absent	Absent	Increased	Increased	Perforation	0.77	No
11	31	Female	1.3	5mm	UTMS	45%	Present	Present	Decreased	Decreased	Macula	0.47	No
12	42	Male	1	6mm	UTMS	50%	Present	Present	Decreased	Decreased	Nebula	0.77	No
13	24	Male	1	4mm	UTMS	40%	Present	Absent	Decreased	Decreased	Nebula	0.47	No
14	46	Male	0.77	3mm	UTMS	20%	Present	Present	Decreased	Decreased	Nebula	0.47	No
15	33	Female	1	3mm	UTMS	30%	Present	Present	Decreased	Decreased	Nebula	0.47	No
16	43	Male	1	5mm	UTMS	50%	Present	Absent	Decreased	Decreased	Macula	0.6	No
17	45	Male	1	6mm	UTMS	50%	Present	Present	Decreased	Decreased	Nebula	0.47	No
18	31	Male	0.77	4mm	UTMS	25%	Present	Absent	Decreased	Decreased	Nebula	0.47	No
19	30	Female	0.77	3mm	UTMS	20%	Present	Present	Decreased	Decreased	Nebula	0.77	No
20	46	Male	1.3	5mm	UTMS	45%	Present	Present	Decreased	Decreased	Macula	0.77	No
21	39	Male	1	4mm	UTMS	40%	Present	Absent	Decreased	Decreased	Nebula	0.77	No
22	47	Male	1.3	4mm	UTMS	30%	Present	Present	Decreased	Decreased	Nebula	0.77	No
23	45	Female	1.3	5mm	UTMS	45%	Present	Present	Increased	Increased	Perforation	0.47	No
24	48	Male	1	3mm	UTMS	30%	Present	Present	Decreased	Decreased	Nebula	0.47	No
25	44	Male	0.77	3mm	UTMS	20%	Present	Present	Decreased	Decreased	Nebula	0.6	No
26	42	Male	1	4mm	UTMS	35%	Present	Present	Decreased	Decreased	Nebula	0.77	No
27	43	Male	1	6mm	UTMS	50%	Present	Present	Decreased	Decreased	Nebula	0.47	No
28	32	Male	1	4mm	UTMS	40%	Present	Absent	Decreased	Decreased	Nebula	0.47	No
29	41	Male	1	3mm	UTMS	30%	Present	Present	Decreased	Decreased	Nebula	0.77	No
30	31	Female	1	**5mm**	UTMS	**50%**	Present	Absent	Decreased	Decreased	Macula	0.47	No

NATAMYCIN ADVERSE EFFECTS										
S.no	**Irritation**	**Itching**	**Headache**	**Watering**	**Redness**	**Sneezing**	**Fever**	**Rashes**	Swelling	Yellow Eye
1	1	1	1	1	1	0	0	0	0	0
2	1	0	0	1	1	0	1	0	0	0
3	1	1	1	1	1	1	2	1	0	0
4	1	1	0	1	1	1	2	1	0	0
5	1	1	1	1	1	0	2	1	1	0
6	1	1	2	1	1	1	1	0	0	0
7	1	1	2	1	1	0	0	0	1	0
8	1	1	2	1	1	1	1	1	0	0
9	1	1	1	1	1	1	2	1	0	0
10	1	1	1	1	1	0	2	1	0	0
11	1	1	2	1	1	0	1	0	0	0
12	1	1	1	1	1	1	1	2	0	0
13	1	1	1	1	1	0	2	1	1	0
14	1	1	2	1	1	1	2	1	0	0
15	1	1	1	1	0	1	0	0	0	0
16	1	1	2	1	1	0	1	0	0	0
17	1	1	1	1	1	1	1	0	1	0
18	1	1	1	1	1	1	1	1	0	0
19	1	1	2	1	1	1	2	1	0	0
20	1	1	2	1	1	0	1	0	0	0
21	1	1	1	1	1	0	2	1	1	0
22	1	1	2	1	1	1	1	1	0	0
23	1	1	2	1	1	0	1	0	0	0
24	1	1	1	1	0	1	0	0	0	0
25	1	1	2	1	1	1	2	1	0	0
26	1	1	1	1	1	1	2	1	0	0
27	1	1	1	1	1	1	1	0	1	0
28	1	1	1	1	1	0	2	1	1	0
29	1	1	1	1	0	1	0	0	0	0
30	1	1	2	1	1	0	1	0	0	0

VORICONAZOLE MASTER CHART													
S.No	Age	Gender	Vision	Epithelial Defect	Infiltrate size	Ulcer depth	Hypo pyon	Satellite Lesion	End of first month	End of second month	End of third month	Vision	Relapse
1	47	Male	1.3	4mm	UTMS	30%	Present	Present	Decreased	Decreased	Macula	1.77	No
2	48	Male	1	2mm	UTMS	20%	Present	Present	Decreased	Decreased	Macula	0.77	No
3	39	Male	1.7	4mm	UTMS	45%	Present	Absent	Decreased	Decreased	Nebula	1.3	No
4	41	Female	1.4	6mm	UTMS	50%	Present	Present	Increased	Increased	Perforation	1.9	No
5	49	Male	1.4	5mm	UTMS	45%	Present	Present	Decreased	Decreased	Nebula	1.4	No
6	31	Female	1.1	3mm	UTMS	45%	Absent	Present	Decreased	Decreased	Macula	0.77	No
7	47	Male	1	4mm	UTMS	30%	Present	Present	Decreased	Decreased	Macula	0.77	No
8	44	Male	1.4	5mm	UTMS	30%	Present	Absent	Decreased	Decreased	Nebula	1.3	No
9	24	Male	1.8	3mm	UTMS	35%	Present	Present	Increased	Increased	Leucoma	1.7	No
10	46	Male	1.3	5mm	UTMS	45%	Present	Present	Increased	Increased	Leucoma	0.7	No
11	29	Male	1.7	3mm	UTMS	40%	Present	Present	Decreased	Decreased	Macula	1	No
12	32	Male	1.3	4mm	UTMS	45%	Present	Absent	Increased	Increased	Leucoma	0.77	No
13	41	Male	1	6mm	UTMS	50%	Present	Present	Increased	Increased	Perforation	2	No
14	43	Male	1	5mm	UTMS	25%	Present	Present	Decreased	Decreased	Nebula	0.77	No
15	37	Male	1.7	3mm	UTMS	30%	Present	Present	Decreased	Decreased	Macula	1.4	No
16	41	Female	1.7	4mm	UTMS	45%	Present	Present	Decreased	Decreased	Nebula	0.77	No
17	44	Female	1	5mm	UTMS	40%	Absent	Present	Decreased	Decreased	Macula	0.47	No
18	27	Male	1.3	3mm	UTMS	35%	Present	Present	Increased	Increased	Leucoma	1.7	No
19	49	Male	0.77	6mm	UTMS	30%	Present	Present	Decreased	Decreased	Nebula	0.6	No
20	43	Female	1	2mm	UTMS	25%	Present	Present	Decreased	Decreased	Nebula	0.77	No
21	42	Male	1.8	4mm	UTMS	45%	Present	Present	Decreased	Decreased	Nebula	1	No
22	34	Male	1.7	3mm	UTMS	30%	Present	Absent	Decreased	Decreased	Nebula	1.3	No
23	46	Male	1	5mm	UTMS	50%	Present	Present	Increased	Increased	Macula	1.3	No
24	41	Male	1.3	3mm	UTMS	35%	Present	Present	Increased	Increased	Leucoma	1.3	No
25	48	Male	1	3mm	UTMS	30%	Present	Absent	Decreased	Decreased	Macula	1	No
26	47	Male	1.3	4mm	UTMS	45%	Present	Present	Increased	Increased	Perforation	1.8	No
27	36	Male	1	3mm	UTMS	30%	Present	Present	Decreased	Decreased	Macula	0.6	No
28	38	Male	1.7	2mm	UTMS	30%	Present	Absent	Decreased	Decreased	Nebula	0.77	No
29	47	Female	1.3	5mm	UTMS	45%	Present	Present	Decreased	Decreased	Nebula	0.77	No
30	45	Male	1.7	4mm	UTMS	45%	Present	Present	Decreased	Decreased	Nebula	1	No

VORICONAZOLE ADVERSE EFFECTS										
S.no	**Irritation**	**Itching**	**Headache**	**Watering**	**Redness**	**Sneezing**	**Fever**	**Rashes**	Swelling	Yellow Eye
1	1	1	2	1	1	1	2	2	0	0
2	1	0	2	1	1	1	2	2	0	0
3	1	1	2	1	1	1	3	1	0	0
4	1	1	2	1	1	0	2	1	0	0
5	1	1	1	1	1	0	1	1	0	0
6	1	1	1	1	1	0	1	1	0	0
7	1	1	1	1	1	0	1	1	0	0
8	1	0	0	1	1	1	1	1	0	0
9	1	1	0	1	1	1	1	1	0	0
10	1	1	1	1	1	0	0	1	0	0
11	1	0	1	1	1	0	0	2	0	0
12	1	1	1	1	1	0	0	2	1	0
13	1	1	1	1	1	0	0	2	1	0
14	1	1	1	1	0	1	1	0	1	0
15	1	2	2	1	2	1	1	0	1	0
16	1	1	2	1	2	1	1	0	1	0
17	1	2	1	1	0	0	1	0	1	0
18	1	2	1	1	0	0	1	0	0	0
19	1	1	1	1	1	1	0	0	0	0
20	1	1	1	1	1	1	0	0	0	0
21	1	1	0	1	1	1	0	1	0	0
22	1	0	0	1	2	0	0	1	0	0
23	1	1	1	1	0	0	0	1	0	0
24	1	1	1	1	0	1	0	1	0	0
25	1	1	1	1	0	1	0	0	0	0
26	1	0	1	1	1	1	1	0	1	0
27	1	1	1	1	1	0	1	0	1	0
28	1	1	1	1	0	2	0	0	1	0
29	1	1	2	1	2	1	0	1	1	0
30	1	1	2	1	1	1	0	1	0	0

ABBREVIATIONS

ABBREVATIONS

FK	FUNGAL KERATITIS
VI	VISUAL IMPAIREMENT
VA	VISUAL ACUITY
AC	ACQUEOUS CHAMBER
KOH	POTASSIUM HYDROXIDE
SDA	SABOURAUDS DEXTROSE AGAR
PCR	POLYMERASE CHAIN REACTION
AE	ADVERSE EFFECTS
MAR	MINIMUM ANGLE OF RESOLUTION
CFCF	COUNT FINGER CLOSE TO FACE
HM	HAND MOVEMENTS
PK	PENETRATING KERATOPLASTY
FDA	FOOD AND DRUG ADMINISTRATION
AREDS	AGE RELATED EYE DISEASE STUDY
HEDS	HERPETIC EYE DISEASE STUDY
CTCAE	COMMON TERMINIOLOGY CRITERIA FOR ADVERSE EVENTS

ETHICAL CLEARANCE LETTER

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Chennai, Tamil Nadu)

ETHICS COMMITTEE CERTIFICATE

Name of the Candidate	:	Dr.S.Prasanna kumari
Course	:	PG in MD., Pharmacology
Period of Study	:	2015 - 2018
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	Study of efficacy and Tolerability of topical voriconazole versus Natamycin in the treatment of filamentous fungal keratitis
Ethical Committee as on	:	24.12.2016

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

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This is to certify that this dissertation work titled **STUDY OF EFFICACY AND TOLERABILITY OF TOPICAL VORICONAZOLE VERSUS NATAMYCIN IN THE TREATMENT OF FILAMENTOUS FUNGAL KERATITIS** of the candidate **Dr.S.PRASANNAKUMARI** with registration Number **201516102** for the award of **M.D.,** in the branch of **PHARMACOLOGY**. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **0** percentage of plagiarism in the dissertation.

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